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<i>Embryological Aspects of Hybrid Vigor in Pines:</i> PROFESSOR JOHN T. BUCHHOLZ	135
<i>Obituary:</i> Nevin M. Fenneman: DR. RAYMOND WALTERS. Recent Deaths	142
<i>Scientific Events:</i> <i>The Professional Training of Chemists; The American Society of Mechanical Engineers; Letters from Scientific Men Abroad; The Retirement of Professor Guyer of the University of Wisconsin</i>	143
<i>Scientific Notes and News</i>	145
<i>Special Articles:</i> <i>Concerted Antibiotic Effect of Penicillin, Methionine, Threonine and Methionine Sulfoxide upon Brucella, Eberthella, Salmonella and Shigella:</i> DR. GREGORY SHWARTZMAN. <i>The Benzyl Ester of Penicillin:</i> DR. M. L. TAINTER and OTHERS. <i>The Functional Pathology of Frostbite and the Prevention of Gangrene:</i> DR. KURT LANGE, DR. LINN J. BOYD and DR. LEO LOEWE. <i>Action Spectrum for the Photoperiodic Control of Floral Initiation in Biloxi Soybean:</i> DR. M. W. PARKER, DR. S. B. HENDRICKS, DR. H. A. BORTHWICK and DR. N. J. SCULLY. <i>A New Polysaccharide from Black Spruce (Picea Mariana):</i> DR. F. E. BRAUNS	148
<i>Scientific Apparatus and Laboratory Methods:</i> <i>A Simple Water Manometer for Recording Intestinal Activity:</i> DR. STEPHEN KROP and DR. TED A. LOOMIS. <i>Use of a Double-Nozzled Spray Apparatus for the Application of DDT or Oils:</i> DR. DONALD F. STARR. <i>Marking Anopheles Mosquitoes with Fluorescent Compounds:</i> DR. JOHN W. ZUKEL	155
<i>Discussion:</i> <i>Antibacterial Action of Quinones:</i> DR. C. A. BROWNE. <i>Green Color of Plant Ash Due to Manganese, Not to Cobalt:</i> W. O. ROBINSON. <i>The</i>	

<i>Color Reaction of Vitamin A on Acid Earths:</i> DR. GERDA GERNSHEIM MAYER and DR. HARRY SOBOTKA. <i>Correction on "Chronic Intermittent Anoxia . . .":</i> PROFESSOR WARD C. HALSTEAD. <i>How Stentor Anchors Itself:</i> PROFESSOR E. A. ANDREWS. <i>The Threat of Anti-vivisection:</i> DR. A. V. WOLF. <i>The Mather Collection of Portraits:</i> DR. T. S. PALMER. <i>The Houssay Journal Fund:</i> DR. HERBERT M. EVANS, DR. WALTER B. CANNON, DR. JOHN F. FULTON and DR. CARL J. WIGGERS	157
<i>Scientific Books:</i> <i>Statistical Analysis:</i> DR. C. I. BLISS. <i>Antibiotic Agents:</i> DR. CHESTER S. KEEFER. <i>Weeds:</i> DR. H. A. GLEASON. <i>Books Received</i>	161
<i>Science News</i>	10

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EMBRYOLOGICAL ASPECTS OF HYBRID VIGOR IN PINES¹

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THE hybrids between some species of pine afford excellent examples of hybrid vigor in the F_1 generation of the cross. During recent years, demonstrations of this hybrid vigor in pines have been made in the nursery of the Institute of Forest Genetics at Placerville, California. The hybrids concerned are not the result of crosses between inbred strains of a species but are the hybrids between species and between varieties. Hybrid vigor has been observed in F_1 plants from the crosses: *Pinus monticola* \times *P. strobus*; *P. jeffreyi* \times (*P. jeffreyi* \times *P. coulteri*) the latter a natural hybrid; *P. murryana* \times *P. banksiana*;

¹ Contribution in lieu of his address as Vice-president and Chairman of Section G (Botanical Sciences) for 1942, American Association for the Advancement of Science.

between certain geographic varieties or races of *P. ponderosa* and in other combinations. In seedling stages, some hybrids have exceeded the growth of the parent species by an amount that appears to be much greater than that of maize. Righter² has given a few performance records over a 3-4 year period of some of these hybrids, which have greatly exceeded the wind-pollinated parents.

The possible manifestation of hybrid vigor during development of the embryo became a subject of special interest to the writer, while he was visiting investigator at the Institute of Forest Genetics, for several months during the summers of 1942 and 1944.

² F. I. Righter, *Jour. Forestry*, 43: 131-137, 1945.

During these periods, investigations were carried out on a variety of embryological subjects, including the causes of sterility in attempted crosses between certain species of pines, on the differences in the embryogeny in large *vs.* small seeds found on the same tree or within the same cone of *Pinus ponderosa* and on the subject of the present account. For the study of the development of hybrid embryos, the cross pollination *P. Murryana* × *P. Banksiana* was repeated in the spring of 1943 so that a series of cones bearing the F₁ embryos during their development could be made available for this study during July and August, 1944.³

It is not necessary to discuss here the numerous investigations on hybrid vigor. Jones⁴ has reviewed the early literature on this subject, while East⁵ has given a critique which applies especially to the genetic aspects of Ashby's hypothesis. Very recently Whaley⁶ has reviewed and discussed the literature on this subject. I am concerned here only with a possible morphological manifestation of this phenomenon which has been claimed in the embryo.

The results of these investigations on the cross *P. Murryana* × *P. Banksiana* have provided a very satisfactory answer to one aspect of the subject of hybrid vigor which has occasioned much discussion, namely, "Is hybrid vigor due, as claimed by Ashby,⁷ to an embryo that is larger at the beginning," the embryo found within the ripe seed. Ashby contends that the embryo of the seed in maize is already larger than the embryos of the parents, so that the seedling plants coming from the germination of the seeds need only to maintain this advantage in size throughout their period of growth, to account for the larger size attained by the hybrid.

It was known⁸ even before Ashby's investigations that the embryos of ripe hybrid seeds are frequently much larger than the embryos of the parent inbred strains. The stress which Ashby places upon the larger size of the embryo is emphasized in his conclusion that this large size of the embryo represents a larger "initial capital." As he states^{7, 9}: "Hybrid vigor in these strains (of maize) is nothing more than the maintenance of an initial advantage in embryo

size." In other words, the "capital" of dividing cells is greater in the hybrids, but the growth rate or compounded "rate of interest" remains the same as that of the higher of the parents.

In a later contribution Ashby¹⁰ modified his definition of "initial capital" to consist of the meristem, presumably the promeristem of the embryo, not including cotyledons. If this is admitted, one would expect to find a more vigorous growing point, a larger promeristem or more meristematic areas in an embryo possessing hybrid vigor. Efforts have been made, therefore, in more recent investigations to measure and compare these growing points in hybrids and in the parent inbred strains. Whaley¹¹ made observation on the promeristem of tomatoes. Murdoch¹² and Shafer,¹³ who designated these meristematic parts as "embryo axis," dissected out the part that included the promeristem of the root and stem tips in the embryo of maize. Though some investigators have reported data that support Ashby's thesis, the repetition of his work on maize by Sprague¹⁴ and the results of the study of the embryo axis by Shafer¹³ lead to conclusions that do not support his hypothesis. However, they seem to lack something in conclusiveness, which may be due very largely to the possible inadequacy of the methods of sampling.

In an effort to test Ashby's thesis Stockwell¹⁵ made a very extensive series of observations¹⁷ on the embryos of *Pinus Lambertiana* and *P. Jeffreyi*, both of which have very large seeds. The seeds were graded for uniformity in size, attached to cards and recorded on x-ray shadowgraphs, then planted in the nursery and tested for growth vigor. The dimensions of the embryos and their anatomical parts could be measured from the enlarged photographs. No significant correlation was found between any of the embryo measurements and the relative size of the seedlings grown from them.

It seems to me a curious circumstance that in none of these studies has the embryo been studied during its development. All the investigations on maize as well as those on tomatoes and other plants begin with a study of the seed and the resulting seedling. Of course a seed consists of an embryo, which may or may not be surrounded or accompanied by endosperm, enclosed within a testa or seed coat. To begin the study in the seed is, therefore, to begin with an embryo, with what one might call a mature embryo. But the embryo of any seed has a definite history of

³ The writer is greatly indebted to the geneticist, F. I. Richter, and W. C. Cumming for making the pollinations, to the director, Dr. Palmer Stockwell, Superintendent R. H. Weidman and others of the staff of the Institute of Forest Genetics for the facilities of the laboratories at Placerville, also to Mr. F. H. Wang, who served as his assistant during the summer of 1944.

⁴ D. F. Jones, *Conn. Agr. Exp. Sta. Bull.*, 207: 5-100, 1918.

⁵ E. M. East, *Genetics*, 21: 375-397, 1936.

⁶ W. Gordon Whaley, *Bot. Rev.*, 10: 461-498, 1944.

⁷ E. Ashby, *Ann. Bot.*, 44: 457-467, 1930.

⁸ T. A. Kiesselbach, *Neb. Agr. Exp. Sta. Res. Bull.* 33, 1926.

⁹ E. Ashby, *Ann. Bot.*, 46: 1007-1032, 1932.

¹⁰ *Idem*, *Ann. Bot.*, N.S. 1: 11-41, 1937.

¹¹ W. Gordon Whaley, *Am. Jour. Bot.*, 26: 609-619, 620-690, 1939.

¹² H. A. Murdoch, *Jour. Hered.*, 31: 361-363, 1940.

¹³ John Shafer, Jr., *Am. Jour. Bot.*, 31: 503-506, 1944.

¹⁴ G. F. Sprague, *Jour. Agr. Res.*, 53: 819-830, 1936.

¹⁵ Palmer Stockwell, *Am. Nat.*, 76: 431-432, 1942.

development, the real embryogeny. As far as I can find out, this period of development has not been touched upon in any investigation having to do with hybrid vigor. There are many investigations that have been concerned with hybrid vigor, not with Ashby's hypothesis but with other phases of this subject, which are not considered here.

The writer feels convinced that pines offer a better opportunity for the study of the embryological aspects of hybrid vigor than plants belonging to the angiosperms. There are, however, some differences which should be pointed out. In the gymnosperms to which pines belong, the megagametophyte, which later serves as the endosperm of the seed, is formed before fertilization of the egg. The endosperm tissue is not derived from the fertilization of a polar fusion nucleus of an embryo sac by the second sperm nucleus. This tissue originates long before fertilization and the genetic constitution of the endosperm is not determined by nuclear fusion. The tissue which forms endosperm in pines is haploid before fertilization and remains haploid subsequently when it assumes the function of endosperm. In angiosperms, especially in the "albuminous" type of seeds such as maize, there is the possibility of hybrid vigor affecting not only the embryo, but also the endosperm, which is triploid tissue, made up of cells with nuclei composed of two maternal sets of chromosomes plus the paternal set contributed from the pollen tube. Some morphologists have avoided the use of the term endosperm in connection with gymnosperms, preferring to designate this tissue female gametophyte or megagametophyte. However, as a physiological term there can be no occasion for confusion; this tissue performs the same function in the seeds of both kinds of plants.

There is, however, another important difference between gymnosperms and angiosperms. The seed of a pine is full grown and has reached its ultimate size at the time of fertilization. This applies without known exception to the pine family and to nearly all conifers. The seed coat becomes stony near the time of fertilization and there can be no enlarging effect on seed size due to the activities of the contained embryo. If fertilization fails or the embryo dies subsequently, the seed coat remains fully as large as when fertilization has taken place. An abortive seed may be full grown, an empty shell complete with wing, but with the contents shriveled. Under such conditions, the immediate internal environment surrounding the developing embryo, whether it be of hybrid origin or from self or wind pollination within the species, is precisely the same.

The ovules and seeds of angiosperms are different in this respect. The phenomenon of double fertilization has already been mentioned, through which the

cells of endosperm tissue have triploid nuclei, two sets of chromosomes coming from the maternal parent and one set derived paternally. The endosperm as well as the embryo come from the fusion of the two male nuclei of a pollen tube with nuclei of the embryo sac, so that we must consider a possible competition between embryo and endosperm, or the combined effect of both in stimulating the growth of the seed.

The complexity of the embryogeny in angiosperms is shown most clearly in the cases of interspecific hybridizations which fail during the development of the seed, after an embryo has begun to develop. Many such instances are known. For example the investigations of Brink and Cooper¹⁶ have given evidence of a lack of nutritional balance between diploid embryos whose nutrition is dependent upon triploid endosperm, which is in turn dependent upon the diploid maternal tissues of the ovule—nucellus and integument. While some of these difficulties are avoided in gymnosperms, there are barriers to hybridization in pines which the writer will describe elsewhere.

At the time of fertilization, the ovule of an angiosperm is small. This organ enlarges greatly after this event due to the stimulus of its contents—a rapidly developing endosperm and an embryo. While the ovule may be regarded as an organ of determinate growth as it develops into a seed, there is at least the possibility that it may become larger if it contains a very vigorous embryo and endosperm than if it contains a weak embryo or an endosperm tissue in some way imperfect. Furthermore, if the ovule of an angiosperm becomes aborted it usually remains small and undeveloped. These facts are so well known as to require no additional elaboration.

However, an important difference between pines and angiosperms, one which is of peculiar advantage in this investigation of hybrid embryos, is the fact that fertilization occurs on nearly the same day within all cones of a tree and in other trees of the same species growing on the same site. Hofmeister¹⁷ first pointed this out in 1851. Strasburger¹⁸ also mentions the fact that certain conifers of central Europe pass through the stages of fertilization with such great rapidity and uniformity in all trees of a forest that he advises the necessity of making daily collections if an event such as fertilization is to be observed. This is all the more remarkable when one realizes that in pines pollination has taken place 13–14 months previ-

¹⁶ R. A. Brink and D. C. Cooper, *Genetics*, 26: 487–505, 1941; *Records, Gen. Soc. of Am.*, No. 12, 1943; D. C. Cooper and R. A. Brink, *SCIENCE*, 95 (2455), 75–76, 1942.

¹⁷ William Hofmeister, "Higher Cryptogamia and Fructification in the Coniferae" (Trans. from German, 1851), Ray Soc. V. 22, London, 1862.

¹⁸ E. Strasburger, "Das Botanische Practicum," Ed. 3, p. 517, Jena, 1897.

ously. In most angiosperms in which fertilization takes place a few hours or days after pollination the time of this event differs from flower to flower; new flowers may open for pollination over a long period.

Fertilization in pines takes place within a period of several days and the date of this event ± 2 days may be determined. We may know, therefore, that the embryos within any two sets of cones collected from a given tree for comparison a month later, are 30 ± 2 days past fertilization, regardless of differences in the sizes of the embryos.

There are other important differences between conifers and angiosperms that should be mentioned. Pine seeds always pass through a history of polyembryony.¹⁹ Though the mature seed crop usually includes fewer than 2 per cent. seeds that contain more than a single embryo, they pass without exception through a stage in the embryogeny in which at least four times this number of embryos are present in the developing seed. Actually there are for a time 8 potential embryos from each egg, when the belated products of the rosette cells are counted.

The writer has examined over a period of more than 25 years embryos of more than two dozen species of pines belonging to various sections of the genus. Their embryogenies are very similar, so much so that it has not seemed necessary to describe them individually. In the pines that have been described,^{17, 19} emphasis has been on the early stages of the embryogeny, in which suspensors are forming and numerous embryos are in a state of competition with each other. There is no known species without this kind of embryonic selection¹⁹ in the early embryogeny. Cleavage polyembryony appears to be universal in pines.

In a study comparing the development of hybrid embryos with non-hybrids, the pines have distinct advantages over angiosperms and no disadvantage, save possibly the long time that must elapse between pollination and fertilization. However, the present study is concerned only with the later stages of the embryogeny, with the largest embryo of each ovule, which may be expected to survive to the maturity of the seed. In this comparison, the embryos that are developing in the seeds of one set of pine cones must be compared with the embryos in the seeds of another group of pine cones at selected times a month or more after fertilization.

The embryos when examined a month to 6 weeks after fertilization finally reach a growth interval during which they enlarge rapidly and pass through a period of differentiation of embryonic organs. This is accompanied by a series of successive morphological

growth stages that may be easily recognized by external appearance during dissection under a binocular microscope. As soon as the stem tip primordium is visible on the end of the dome-tipped meristematic embryonic cylinder, borne in turn on the end of a long twisted suspensor, a definite stage of development, Stage III, may be recognized. Fig. 1 represents the external appearance and relative sizes of the embryos during various stages. The plumule primordium not only stands out as a small dome or cone at the tip of the larger dome, but this beginning of a stem tip is recognizable during the dissection of living embryos¹⁹ by a greater translucency when observed under oblique transmitted light. The dotted line in Fig. 1, Stages II, III, etc., represents an internal arrangement of cells at the root tip, the more massive cylinder below this is essentially the root cap, merging into the suspensor. Only the spherical part of the embryonic cylinder between the dotted line and the tip eventually contributes any of the parts of the embryo that appear above ground in the seedling. The embryos found in other seeds of the cone, that are smaller and have not reached Stage III, have been classified for convenience into two arbitrary size classes, Stages I and II. These stages are of long duration since Stage I may include some embryos that would not become Stage III for more than a week.

Stage IV follows Stage III closely within a day or less than two days. The embryo is now somewhat larger and has a circle of cotyledonary primordia surrounding the stem tip primordium. The number of cotyledonary primordia may usually be counted and recorded by patiently rotating the embryo. These primordia are also more translucent under oblique transmitted light when living embryos are examined.

Stage V is an embryo 1-2 days older than the previous stage and therefore larger, defined as the stage in which the cotyledons have enlarged sufficiently to be equal in length to the stem tip primordium. This stage is easily recognized.

Stage VI includes embryos about 1-2 days older than Stage V, in which the cotyledons definitely exceed the stem tip in length on all sides, but are less than twice as long. The cotyledons do not hide the stem tip completely, which may still be observed by tilting the end of the embryo toward the observer.

The four Stages III-VI, with an average duration between 1-2 days each, may be considered as very definite morphological growth stages by means of which relative maturity of the embryos in the seeds of one pine cone or group of cones may be compared with those of another collection of cones, either from the same tree or from other trees. Such stages are comparable in a comparison of the embryos coming from seeds of different sizes; they make it possible

¹⁹ J. T. Buchholz, *Bot. Gaz.*, 66: 185-228, 1918; *Idem.*, *Bot. Gaz.*, 73: 249-286, 1922; *Idem.*, *Trans. Ill. Acad. Sci.*, 23: 117-125, 1931.

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to compare the relative stages of development of embryos of different species of pines.

Stages VII and VIII are two somewhat arbitrary size classes into which the embryos that are more mature than VI may be separated. In Stage VII, the stem tip is completely obscured by the cotyledons. Stages VII and VIII are of much longer duration than the previous stages. Stage VII may be of only one or a few days longer duration than Stage VI, but Stage VIII may include full-grown embryos, static for several weeks or a month, until the seed is shed. However, it is very probable that seeds with embryos in this stage, even some in Stage VII, are ready for harvest and would germinate if properly extracted.

embryos have been dissected from seeds of all possible sizes found on six different individual trees of *Pinus ponderosa*. The size of the seed has an influence on the size of the embryo at the time when Stages III-VII are attained. These stages are not determined only by the number of cells or volume in the embryo itself; for in a large seed, an embryo may have a volume more than three times as large as in a small seed when it is in any of the stages III-VI, corresponding somewhat but not fully to the difference in volume of the seeds.

There was a difference in the sizes of the seeds in the two species of pines that are compared here. The seeds of *Pinus Banksiana* are mostly No. 6 and No.

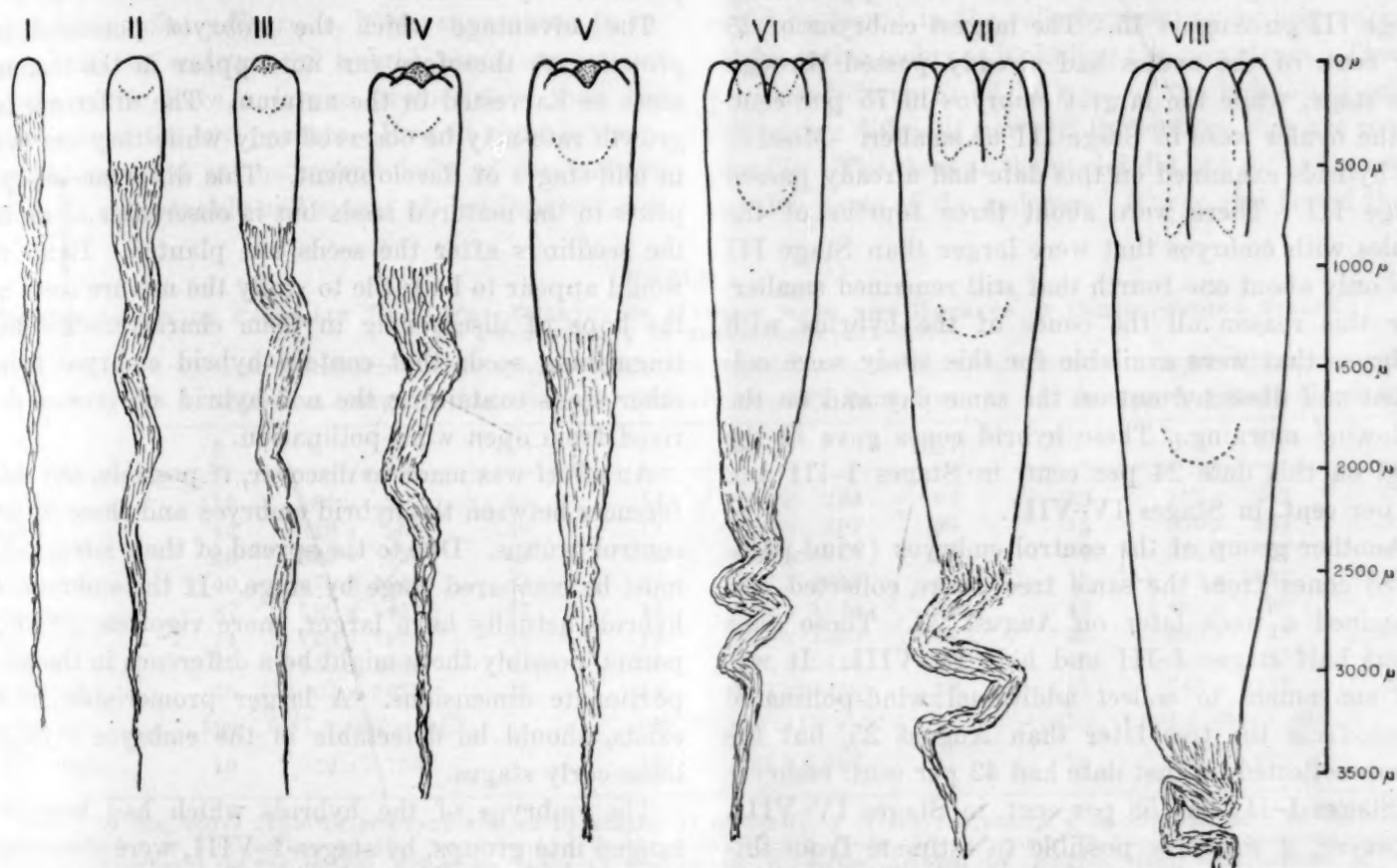


FIG. 1. Stages in differentiation of stem tip primordium and cotyledons in a pine. The figures were made to approximate scale of the means of the embryos: *Pinus Murryana* × *P. Banksiana*, as observed 35 days after fertilization. The plerome of the root tip shown by dotted line may be observed only in sections beginning with Stage II. The cylinder of tissue below the dotted line is mostly root cap, from the lower portion of which cells have elongated to form the suspensor, so long that it is coiled and twisted within the endosperm. The stem tip primordium which first appears in Stage III is shaded and is indicated by dotted line in Stages VI-VIII.

The size ultimately attained by the embryo is limited by the size, mainly the length, of the cavity hollowed out within the endosperm and this in turn by the size of the seed, whose full dimensions are reached at the time of fertilization. The growth of the embryo is arrested soon after Stage VIII is reached, to be resumed only when the seed is planted.

Elsewhere²⁰ an extensive set of measurements of the embryos is given by stages as defined here. These

²⁰ J. T. Buchholz and Mary L. Stiemert, *Trans. Ill. Acad. Sci.*, Vol. 38 (in press).

7, corresponding to widths up to 2.36 mm and 2.77 mm; those of *Pinus Murryana* are No. 7 and No. 8, corresponding in maximum widths up to 2.77 and 3.17 mm. However, in the accompanying summarized table, based upon means of all stages, embryos coming from seeds of all sizes are grouped together. The seed sizes were fairly well distributed among the stages.

The trees of *Pinus Banksiana* which furnished the pollen for this hybrid experiment were growing in the Eddy Arboretum at Placerville. At this elevation of

2,740 feet, fertilization and the various stages of the embryo are passed through more than a month earlier than at altitudes above 5,500 feet. The embryos dissected from the cones of the pollen parents were obtained during July in all stages I-VIII.

The maternal parent, a single tree in a group of *P. Murryana*, was growing on the west slope of the Sierras near Strawberry, along highway 50 at 5,700 feet elevation. The same tree furnished the embryos of the controls from wind pollination. During 1944 fertilization in this tree occurred between July 10 and 12.

It was found from examination made from time to time upon the wind-pollinated cones of this tree at Strawberry that the embryos were approaching Stage III on August 15. The largest embryos of 25 per cent. of the ovules had already passed through this stage, while the largest embryos in 75 per cent. of the ovules were in Stage III or smaller. Most of the hybrids examined on this date had already passed Stage III. There were about three fourths of the ovules with embryos that were larger than Stage III and only about one fourth that still remained smaller. For this reason all the cones of the hybrids with embryos that were available for this study were collected and dissected out on the same day and on the following morning. These hybrid cones gave in the total on this date 24 per cent. in Stages I-III and 76 per cent. in Stages IV-VIII.

Another group of the control embryos (wind-pollinated cones from the same tree) were collected and examined a week later on August 22. These gave about half stages I-III and half IV-VIII. It was not convenient to collect additional wind-pollinated cones from the tree later than August 25, but the group collected on that date had 42 per cent. embryos in Stages I-III and 58 per cent. in Stages IV-VIII. However, it was now possible to estimate from this that the embryos on this tree from wind-pollination would have reached the stages with 24 per cent. I-III vs. 76 per cent. IV-VIII, by August 28-30. It was possible to conclude that about two weeks longer was required for the embryos of the wind-pollinated control to reach the same mid-stages of development than for the embryos of the hybrid. This is a remarkable difference, especially when we consider this divergence in terms of the number of days after fertilization. Three fourths of the embryos of the hybrids reached the stages IV-VIII in 35 days after fertilization, while this proportion of the embryos of the wind-pollinated controls required as estimated 48-50 days to reach the same distribution in size and stage of development.

This set of observations indicates very definitely that the embryos of the hybrids actually grow much

more rapidly. Naturally one then asks, "What is their relative size at the maturity of the seed?" In the mature seeds these embryos can not be larger than the embryos of the controls. They continue to grow until the space available inside of the endosperm is filled. Probably all the embryos of the hybrids reached their full size by about August 20-25; in fact, many of them had already reached this size on August 15. The controls required another two weeks to reach about the same size distribution but at the maturity of seeds they were actually slightly larger than the embryos in the seeds of the hybrids. There is a margin of several weeks or possibly a month during which any embryos that are still somewhat small may grow to the full size permitted by the seed.

The advantage which the embryos possessed in *growth rate* therefore can not appear in the mature seeds as harvested in the autumn. The difference in growth rate may be observed only while they are still in mid-stages of development. This difference disappears in the matured seeds but is observable again in the seedlings after the seeds are planted. Hence it would appear to be futile to study the mature seeds in the hope of discovering in them characteristics distinguishing seeds that contain hybrid embryos from other seeds containing the non-hybrid *sib* crosses derived from open wind-pollination.

An effort was made to discover, if possible, any differences between the hybrid embryos and those of the control groups. Due to the spread of their sizes, these must be compared stage by stage. If the embryos of hybrids actually have larger, more vigorous growing points, possibly there might be a difference in the proportionate dimensions. A larger promeristem, if it exists, should be detectable in the embryos even in these early stages.

The embryos of the hybrids which had been arranged into groups, by stages I-VIII, were measured. This included the hybrid embryos dissected from a cone on August 8. Likewise the embryos of the control parent, all of which came from wind-pollinated cones borne on the same tree, and those that had been dissected from the pollen parents in the pinetum were also arranged into similar groups by stages and measured. The embryos of hybrids are shown by stages between the parents in Table 1. The final dimensions of a few of the embryos of mature seeds that were harvested in the fall by the usual methods have been added below and indicate how much smaller the various stages are than their ultimate sizes within the ripe seeds.

Table 1 gives the comparison by stages, with the actual mean dimensions in microns. This shows that the embryos of the hybrids stand midway between the embryos of corresponding stages and size classes of

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the two parents. As pointed out earlier the embryos of Stages III, IV, V and VI were classified and determined by rather definite morphological criteria. Those of Stage I and II and the Stages VII and VIII are separations made arbitrarily as size-classes.

In their dimensions and in their shape index (diameter divided by length) the hybrids stand between the corresponding figures obtained for the two parents. This also holds for the cotyledon number which was counted individually for nearly every embryo. The mean number of cotyledons of the hybrids, $5.246 \pm .115$, stands between the numbers shown in the two parents, $5.869 \pm .067$ and $4.59 \pm .050$.

This number might be expected to be larger if the growing point is larger, since the cotyledons surround the growing point. There is, however, nothing to indicate that the embryos of hybrid pines are in any way larger or have a larger promeristem than the mean between the two parents. Hybrid vigor is therefore not reflected in the morphology of the embryo during the comparable mid-stages of development, nor

of the hybrid is stage for stage, between that of its parents during comparable stages of development, in actual dimensions as well as in comparison with an index of shape—the diameter divided by the length.

There was, however, still a possibility that Ashby's thesis might be partially substantiated if the number of cells in the embryos of the hybrids is greater than the number in comparable stages of the parent species. Of course, the embryos of the hybrid have already been shown to be no larger, but it was thought that the cells might be somewhat smaller and therefore more numerous. The embryos were embedded in paraffin, sectioned, some longitudinally, others transversely and stained by the usual methods. When these were examined and carefully compared, no difference could be found in the cells by comparing any region of the entire embryos including the meristems. There were no differences in the sizes of the individual cells composing different parts of the embryo that are comparable. The sizes of the nuclei did not differ in comparable parts of the embryos. It was also found that

TABLE 1
COMPARISON OF PARTIALLY DEVELOPED EMBRYOS OF HYBRIDS WITH THE EMBRYOS OF CORRESPONDING STAGES OF DEVELOPMENT IN THE PARENT SPECIES

	P. Murryana × wind pollination				P. Murryana × P. Banksiana				P. Banksiana × wind pollination			
	f	d	l	d/l	f	d	l	d/l	f	d	l	d/l
Stage I	116	181 ×	227	.80	14	179 ×	269	.67	35	153 ×	242	.63
Stage II	43	310 ×	558	.56	18	293 ×	507	.58	13	250 ×	484	.52
Stage III	26	395 ×	735	.54	12	385 ×	762	.50	8	373 ×	807	.46
Stage IV	40	473 ×	1064	.44	11	454 ×	1050	.43	8	443 ×	1033	.43
Stage V	37	599 ×	1417	.42	14	549 ×	1344	.40	25	483 ×	1290	.37
Stage VI	24	723 ×	1868	.39	12	611 ×	1802	.34	36	568 ×	1728	.33
Stage VII	37	859 ×	2681	.32	21	725 ×	2418	.30	45	624 ×	2261	.28
Stage VIII ...	25	1002 ×	3675	.27	16	884 ×	3339	.26	41	740 ×	2838	.26
Cotyledons	160	$5.87 \pm .067 \sigma .852$			69	$5.246 \pm .115 \sigma .954$			155	$4.594 \pm .050 \sigma .619$		
Embryos												
Ripe seeds ..	10	$1,022 \times 3,759$			12	$993 \times 3,657$			25	$790 \times 3,178$		

NOTE: In the above table of embryonic sizes by stages of maturity, *f* is the frequency, *d* is the diameter of this embryonic cylinder in the region of the cotyledons, *l* is the total length from the suspensor to the tip of the embryo or cotyledons, whichever is the longer, *d/l* is an index of proportion, diameter divided by length. Only the means of all embryos (*f*) belonging to each of the different stages are given, converted to microns from a micrometer scale accurate to about 8 micron units in the original measurements.

is the embryo in the mature seed larger than the non-hybrid embryo of the maternal seed parent.

If the hybrids should have a larger promeristem represented by the plumular growing point, this feature should be shown by the embryo of Stages III-IV. The hybrids should give a different index of shape. There are slight variations here, but they are not significant. The variations shown are probably due very largely to differences in seed size. If the stages III-VI are combined and their mean shape index computed, there is no significant difference in the hybrids from a mean falling between the corresponding mean of the two parent species.

From these data we may conclude that the embryo

the areas that might be included in the promeristem—the primordium of the stem tip surrounded by the circle of cotyledons, did not appear larger or include a larger number of cells.

There is but one significant difference in hybrids that was found by this investigation: the embryos of the hybrid grow more rapidly than those of the parents. Hybrid vigor is not due to a larger embryo in the seed in comparable stages of development nor to meristematic growing points that are larger in the embryo. Hybrid vigor is nothing that may be detected by a morphological study of the embryos themselves. The nature of hybrid vigor is however defi-

nitely a physiological vigor of growth; its real explanation is to be sought in physiological, possibly biochemical investigations.

As pointed out in my introduction Ashby's hypothesis^{7, 9, 10} as he stated it, depends upon greater "initial capital" represented by a larger embryo or larger growing point in the embryo, enjoying the same growth rate, which he expressed as "rate of interest." To restate my results in terms of his analogy, I can

definitely state that hybrid vigor is due to an initial capital which is no larger, stage by stage, than the mean between the parent species, but the embryo enjoys a higher growth rate, or a higher compounded "rate of interest," before the seed is mature as well as after planting. It may be expected that in an angiosperm wherever the hybrid embryo in a seed is larger than that of the parents, this is the result rather than the cause of hybrid vigor.

OBITUARY

NEVIN M. FENNEMAN

DR. NEVIN M. FENNEMAN, professor emeritus of geology of the University of Cincinnati, died in Cincinnati, Ohio, on July 4, 1945, in his seventy-ninth year. In the fields of geology, geography and physiography, his fellow workers in this country and abroad will regret the passing of a distinguished and greatly esteemed colleague.

He was born on December 26, 1865, at Lima, Ohio, a son of William Henry and Rebecca Oldfather Fenneman. He took his undergraduate course at Heidelberg College, Tiffin, Ohio, where he was graduated A.B. in 1883. There followed a period of high-school teaching and eight years, 1892 to 1900, as professor of physical sciences at the Colorado State Normal School, now the Colorado State College of Education. He had his graduate training under Chamberlin and Salisbury at the University of Chicago, receiving the Ph.D. degree in 1901.

Dr. Fenneman became the first professor of geology at the University of Colorado in 1902, at about the time the oil fields in the Boulder area were opening. These fields were of minor commercial importance, but their geologic setting abounded in scientific interest and Dr. Fenneman's report upon them, published in the bulletin series of the United States Geological Survey, attracted considerable attention. He received various assignments from the Geological Survey for work in western and southern states, with resultant publications during these and later years. After three semesters at the University of Colorado, he was called to the University of Wisconsin in 1903, and he served as professor of geology there for four years. From 1900 to 1902 he was geologist of the Wisconsin Geological and Natural History Survey, and, from 1906 to 1908, geologist of the Illinois Geological Survey.

In 1907, Dr. Fenneman came to the University of Cincinnati and established the department of geology and geography. Nature had made the Cincinnati area one of geological significance as the type locality for Ordovician marine deposits. Dr. Fenneman made it a center of scientific importance for instruction and

research in geology and geography. He chose, guided and inspired young members of his department, and they and the students majoring in geology at Cincinnati have accomplished notable professional work. As a departmental colleague expresses it:

Behre, Bucher and a long list of others regard him as the outstanding influence on their lives. His stern teaching, his kindly, searching criticism, his keen encouragement and his own deep humility, all left their mark. Hardly a month goes by but some U.C. graduate, whose only contact with Fenneman may have been in freshman geology, recalls the impact of a mind which taught him to think rigorously and which opened new vistas. . . . He was a master of logical presentation, the last of the great trio of American physiographers—Davis, Fenneman and Johnson—of the early twentieth century, who followed in the steps of the great pioneers in this field and developed a rigid application of logic to the study of land forms and their evolution.

During his thirty years as department head to 1937 and afterward (he was at his campus desk daily and continued his advanced courses during his eight *emeritus* years) Dr. Fenneman slowly and steadily worked on studies which won wide acclaim upon publication. These included numerous reports and bulletins, ranging from a local enterprise—direction of a survey of the natural and industrial resources of the Cincinnati area—to an international project—direction of scientific work on Africa preparatory to the World War I peace conference at Paris. The U. S. Geological Survey, which he served as assistant geologist, associate geologist and geologist for more than a quarter of a century, published his map "Physical Divisions of the United States," which was the original of its type. His *opus magnum* was the "Physiography of Western United States" and "Physiography of Eastern United States," volumes now already classic in this field.

Fellow scientists in his and related fields recognized Dr. Fenneman's achievements in many ways. He was elected president of the Association of American Geographers in 1918; chairman of the National Research Council's division of geology and geography

in 1922; vice-president and chairman of the geology section of the American Association for the Advancement of Science in 1923; president of the Geological Society of America in 1935; president of the Yellowstone-Bighorn Research Association in 1936. Among his international contacts were those made in the Far East in 1926, when he represented the United States at the Pan Pacific Science Congress at Tokyo. His name was starred in editions of "American Men of Science" for the past quarter of a century. "For eminent achievements in the physiography of the United States," Dr. Fenneman was awarded the gold medal of the Geographic Society of Chicago in 1938. He received the honorary degree of doctor of laws of the University of Cincinnati in 1940.

In addition to the societies and associations already indicated, Dr. Fenneman held membership in the American Society of Naturalists, Sigma Xi, Phi Beta Kappa, Ohio Academy of Science, the Cosmos Club of Washington, D. C., and the Literary Club of Cincinnati, of which he was president in 1924-25.

Dr. Fenneman was married in 1893 to Sarah Alice Glisan, who died in 1920. They had no children.

Aside from his scientific interests, Dr. Fenneman followed national and world affairs closely and was long an advocate of an association of nations. He had a lighter side, with flashes of wit and dry humor which were the delight of colleagues and students alike. He wrote numerous familiar essays marked by an individuality and homely flavor which charmed his hearers when he read them as papers at meetings of the famous Literary Club of Cincinnati.

Dr. Fenneman was a tall man of rugged physique who, up to his last weeks, walked with springy step between his Clifton home and the university campus. A portrait, painted recently by Frank H. Myers, the Cincinnati artist, admirably shows his Lincolnian features. His voice was deep and resonant, his speech deliberate, his manner one of courtesy and charm. A stalwart and fascinating personality, he was an eminent figure in the history of the university which he served so loyally and well. Undaunted by

storms, he was like a great oak tree, spreading friendly protection for others. This influence is typified in a letter from a scientist who owed much to him: "He never knew how greatly I admired in him that in which he was great: his iron self-discipline; his sturdy self-sufficiency; his profound urge to grasp fundamentals; his fastidiousness; his rare sense of fitness in the choice of words; and his live sense of humor."

At the funeral service on July 6 in the Mt. Auburn Presbyterian Church, Cincinnati, of which Dr. Fenneman was a member, the Reverend Henry C. Rogers read the passage from "Pilgrim's Progress" relating to Mr. Greatheart and his service to others, concluding: "Mr. Greatheart has returned to his Master."

RAYMOND WALTERS

UNIVERSITY OF CINCINNATI

RECENT DEATHS

DR. AUGUSTUS H. FISKE, chief chemist of the Rumford Chemical Works, retired, died on July 27 at the age of sixty-five years.

DR. GEORGE A. HARROP, JR., of Princeton, N. J., a vice-president of E. R. Squibb & Son and research director of the Squibb Institute for Medical Research, New Brunswick, N. J., has died at the age of fifty-four years.

A LETTER received by Dr. William Randolph Taylor from Dr. F. Børgesen of Copenhagen announces the recent death of Mme. Anna Weber-van Bosse, of Eerbeek, Netherlands. She was a distinguished student of Netherlands East Indian algae whose elaborate reports in the "Siboga Expeditie" are well known, as is her monograph on *Caulerpa* and other works on tropical algae. She had passed her ninetieth birthday at the time of her death.

DR. LEON J. COLE writes that a report has come from the Philippines that Dr. Miguel Manresa, formerly head of the department of animal husbandry in the College of Agriculture at Los Baños, met his death on February 11 before the liberation of Manila.

SCIENTIFIC EVENTS

THE PROFESSIONAL TRAINING OF CHEMISTS

THE American Chemical Society is planning to seek the release for further training of a select group of servicemen who have received college degrees since 1940 and who as students showed unusual promise in science and technology, according to a statement made by Dr. Erle M. Billings, of Rochester, N. Y., secretary of the Committee on the Professional Training of Chemists.

The committee has sent a questionnaire to the departments of chemistry and chemical engineering of colleges and universities throughout the country requesting them to provide "a highly selective list of college graduates since 1940 in certain fields, whose high ability, intelligence and leadership make their continued training at the earliest possible moment of demonstrable value to the country." In addition, the society hopes that some of the best upperclass students may also be discharged so that they may finish

their work for the bachelor of science degree.

When the national roster of young scientific talent is complete, it will be submitted to the armed services with the request that the men be returned to the classrooms in order to make possible "the early resumption of chemical education and training on a proper scale."

"There is some evidence," according to the statement, "that the armed services may be receptive to a properly presented plan for the early return, by assignment or demobilization, of a limited number of especially capable young men to further training." The training of chemists and chemical engineers has been disrupted by the war and threatens our national technological competence. The statement continues:

The American Chemical Society feels impelled to do everything possible to hasten the return to college of potential chemists and chemical engineers whose education was interrupted by the war. Only by insistence on prompt resumption of such training can the war-induced deficits be even partially alleviated.

Two actions can aid in such a program: one, to secure the release of especially capable men from the armed services to permit them to resume their training at the earliest possible date, and the other, to provide financial aid when needed.

Various scholarship funds have been and are being established to help to accelerate training. This makes ability the controlling factor.

The institutions from which the information is sought are on the approved list of the American Chemical Society.

Professor W. Albert Noyes, Jr., University of Rochester, is chairman of the committee. Other members are: Dr. W. G. Young, University of California at Los Angeles, and Dr. H. B. Weiser, the Rice Institute, Houston, Texas.

THE AMERICAN SOCIETY OF MECHANICAL ENGINEERS

THE American Society of Mechanical Engineers has made public the names of those selected to receive its 1945 honors and awards. The formal presentation will be made in New York late in November.

The American Society of Mechanical Engineers Medal, the society's highest honor, awarded annually for distinguished service in engineering and science, has been awarded to Dr. William Frederick Durand, chairman of the division of engineering and industry of the National Research Council, professor of mechanical engineering emeritus of Stanford University. It will be presented in recognition of his work in hydrodynamic and aerodynamic science, particularly the forwarding of the design and application of the principles of jet propulsion; and of his services to the government in engineering research.

The Holley Medal, presented for "some great and unique act of genius of an engineering nature," will

go to Dr. Sanford Alexander Moss, engineer of the General Electric Company, West Lynn, Mass., for his pioneer work in turbosuperchargers which largely made possible the height, speed and range of modern aircraft. Retiring in 1938, Dr. Moss was in London the day of the Munich pact. He returned and, at the age of sixty-seven years, voluntarily resumed work as a consulting engineer for the General Electric Company. On his seventieth birthday, in 1942, Lieutenant General H. H. Arnold sent greetings in behalf of the Army Air Forces, saying: "Your contribution of the airplane supercharger and turbosupercharger is outstanding in the science of aeronautics."

Dr. Joseph M. Juran, assistant to the administrator of the Foreign Economic Administration, Washington, D. C., will receive the Worcester Reed Warner Medal, which is given for noteworthy contributions to engineering literature. It will recognize his contribution to the problem of quality control in mass production and various other writings.

The Melville Prize Medal for an original work will be presented to William Julian King, research engineer with the fuels division of Battelle Memorial Institute, Columbus, Ohio, for his paper, "The Unwritten Laws of Engineering." His work has been concerned chiefly with fundamentals of combustion liquid fuels and the development of gas turbines.

Bruce E. Del Mar, supercharging engineer with the Douglas Aircraft Company, Santa Monica, Calif., will receive the Junior Award for his paper, "The Presentation of Centrifugal Compressor Performance in Terms of Non-Dimensional Relationships."

A later announcement will be made regarding student awards.

Honorary membership in the society has been conferred as follows:

On Wong Wen-hao, of Chungking, China, vice-president of the Executive Yuan and Minister of Economic Affairs and head of the National Reconstruction Commission of the Chinese Government. The award will pay tribute to his preeminence in the field of professional public service.

On Sir William Arthur Stanier, F.R.S., director of scientific research in the Ministry of Production, London. He will be honored for influencing "in an outstanding fashion the technique of railway transport in our present age."

On Rear Admiral Harold Gardiner Bowen, U.S.N., Naval Research Laboratory, Anacostia Station, Washington, D. C., for his service to his country—"his valiant and successful fight to introduce steam of high pressures and high temperatures into the United States Navy. The resulting performance to-day is making naval history."

On Dugald Caleb Jackson, professor emeritus of the Massachusetts Institute of Technology, "for outstanding leadership in education and consulting engineering fields."

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On Andrey Abraham Potter, acting president and dean of engineering at Purdue University, "for leadership in adjusting engineering education to the needs of war training as chairman of the Engineering Science and Management War Training Program of the U. S. Office of Education."

LETTERS FROM SCIENTIFIC MEN ABROAD

PROFESSOR D. WORONZOW, director of the Physiological Institute of the State University of Kiev, writes to Dr. A. J. Carlson, University of Chicago, as follows:

The university buildings, including my laboratory and library, were wrecked and burned. The libraries of the Medical Institute were also destroyed. . . . I am turning for aid to my colleagues in countries friendly to us, asking them to help us in rehabilitating physiology in Kiev. We shall be extremely grateful should you find it possible to contribute to our institute duplicate copies of books and journals from your personal library, as well as reprints of your own papers (and those of your colleagues) that you may have on hand.

Dr. M. Demerec, of the Carnegie Institution, Cold Spring Harbor, New York, states that in a letter mailed on July 14 Professor Ernst Hadorn, Zoologisches Institut der Universität, Zürich, Switzerland, writes: "Since I have not seen any American publication since 1941-42 I would appreciate it very much if my colleagues would send me reprints of their papers as soon as this is possible. We live here in isolation and are anxious to know what is going on in the scientific world."

Professor Marston Taylor Bogert, of Columbia University, has received a letter from Professor W. P. Jorissen, of Leiden (Hooge Rijndyk 15), which reads as follows:

We are very happy to be free again in Holland after five terrible years of oppression, and we hope that the deliverance of our India will follow soon. I am recovering from edema caused by insufficient food, but hope to be able in a few weeks to visit our laboratory again (that for physical and inorganic chemistry) which the Germans robbed of its best instruments and part of its books and journals.

I am longing to know how you are and what the Union

Internationale de Chimie has been able to do in these years of war. Did you see already our Report on the Nomenclature of Inorganic Compounds? Till now I did not receive a copy of Professor Bassett's English version. Nor did I see the Italian version.

You can imagine how much we are in arrears with American chemical literature, but I hope our colleagues in the States will send us reprints of their work. As you know, I am especially interested in oxidation processes and their inhibition and in explosive reactions. Perhaps you have still available reprints of your research on the oxidation of aldehydes.

THE RETIREMENT OF PROFESSOR GUYER OF THE UNIVERSITY OF WISCONSIN

PROFESSOR M. F. GUYER, since 1911 chairman of the department of zoology of the University of Wisconsin, retired on July 1.

Before going to Wisconsin he received his Ph.D. degree at the University of Chicago in 1900 and spent eleven years at the University of Cincinnati.

Under his chairmanship the staff of the department of zoology at Wisconsin has increased about sixfold. In addition to the many hundreds of undergraduate students who have had contact with him as a teacher, one hundred and twenty-five men and women have received Ph.D. degrees in zoology during this time. These men and women are scattered in nearly every state of the union, in Washington, D. C., Hawaii, South America, Canada, China, England, Italy and the islands of the Pacific. Many are teaching in colleges and universities, others are doing research or practising medicine, and still others are serving in the armed forces.

In appreciation of Professor Guyer's long and constant service to the university, seventy-eight former and present members of the department honored him by attending a banquet in Madison on April 21. At the dinner he was presented with a portfolio of letters from most of his former graduate students who were unable to attend. A talent unsuspected by many was discovered when songs of Professor Guyer's own composition were sung as a part of the program.

Being relieved of administrative and teaching duties, Professor Guyer now plans to devote his time to writing and research.

SCIENTIFIC NOTES AND NEWS

THE medal of the American Iron and Steel Institute for 1944, awarded annually for "the outstanding technical paper of the year on steel," has been awarded to Dr. Wendell E. Hess, professor of metallurgical engineering at the Rensselaer Polytechnic Institute.

At a dinner given on July 25 in Washington by the

Variety Clubs of America in honor of Sir Alexander Fleming, the Humanitarian Award was presented to him "as the man in 1944 whose humanitarian efforts had contributed the most to the welfare of mankind." The award consists of a silver plaque and an honorarium of \$1,000. A research fund of \$80,000 for Sir Alexander has been established by American manu-

facturers of penicillin. It will be administered by the University of Pennsylvania. Sir Alexander will conduct the research at St. Mary's Hospital Medical School, University of London.

THE Philadelphia College of Pharmacy and Science at its one hundred and twenty-fourth annual commencement on July 24 conferred the degree of doctor of science, *honoris causa*, on Dr. Robert P. Fischelis, secretary of the American Pharmaceutical Association, with the following citation: "Outstanding exponent and champion of the fundamental good which the profession of pharmacy, quietly but insistently and consistently, brings to the public in its diversified services. In your important work in the War Production Board, you gave your untiring zeal and your wealth of experience so that this nation might earn its day of victory and gain the peace to which democracy is dedicated. Now, as leader of the thinking and the action of the American Pharmaceutical Association, you are exploring and charting new ways to use the coming days of peace for the greatest good to humanity."

W. D. COLLINS was honored on June 20 by a group of friends at the Cosmos Club in Washington in recognition of twenty-five years of service as chief of the Quality of Water Division of the U. S. Geological Survey. He was presented with a petrified-wood desk set and a volume of congratulatory letters from friends and business associates in the Survey and the chemical profession.

THE award of the Alumni Association of Oberlin College for notable service to the college has been presented on the occasion of his retirement to Dr. Harry N. Holmes, since 1914 professor of chemistry and head of the department.

DR. EDWIN C. MILLER, professor of plant physiology at the Kansas State College and plant physiologist of the Kansas Agricultural Experiment Station, will retire on August 31 after thirty-five years of service at that institution. On the evening of May 28 a department dinner was held honoring Dr. and Mrs. Miller at which time administrative officers of the college and experiment station reviewed Dr. Miller's work. President M. S. Eisenhower, of the college, announced that Dr. Miller has been made professor emeritus of plant physiology. A book of more than two hundred letters from former students, colleagues (past and present), contemporary plant physiologists and other friends was presented to Dr. Miller, and a department gift was presented to Dr. and Mrs. Miller. The Edwin C. Miller Scholarship in Plant Physiology was announced. This scholarship of \$200 was provided by his son, Jordan Y. Miller, and daughter, Mrs. Josephine Miller Henderson, and her husband.

Dr. Miller's address after August 1 will be R.F.D. No. 1, Baltimore, Ohio, where he will retire to the farm on which he was born. Dr. John C. Frazier, who has been associated with him since 1936, will succeed him at the Kansas State College and the Kansas Agricultural Experiment Station.

LIEUTENANT HAROLD T. COOK, head of the department of plant pathology of the Virginia Truck Experiment Station at Norfolk, has been on military leave since September 29, 1942. He was stationed in the South Pacific until February 18 of this year. Since then he has been in the United States for a month's furlough, after which he has been on overseas duty in the Mariannas. He received on July 8 the letter of commendation that is given below, signed by Vice-Admiral J. H. Newton, U. S. Navy. "The Commander South Pacific Area and Force takes pleasure in commending Lieutenant Harold Thurston Cook, U. S. Naval Reserve, for service as set forth in the following: For meritorious and efficient performance of duty while serving on the staff of the United States Joint Purchasing Board, South Pacific Area from December 18, 1942, to January 17, 1945. During this period, Lieutenant Cook displayed exceptional ability in handling the many detailed problems incident to the procurement of maximum quantities of food from New Zealand for our forces afloat and ashore. Through his initiative and efficient coordination of the production and procurement programs, he contributed materially to the maintenance of a continuous flow of supplies necessary for the subsistence of our forces during the critical period of operations in the South Pacific. His conduct throughout was in keeping with the highest traditions of the United States Naval Service."

DR. WILLIAM E. WICKENDEN, president of the Case School of Applied Science, was elected president of the American Institute of Electrical Engineers at the annual meeting held in New York on June 27.

WESLEY WINANS HORNER, consulting civil engineer, of St. Louis, has been nominated for president of the American Society of Civil Engineers.

THE retirement on July 1 is announced of Professor Edwin Brett Hart, of the department of agricultural chemistry of the University of Wisconsin.

DR. ISIDOR I. RABI, professor of physics at Columbia University, has been made executive officer of the department.

DR. EARL T. APFEL, professor of geology, has been appointed chairman of the department at Syracuse University, to succeed Dr. George B. Cressey, who resigned recently.

DR. J. MARVIN WELLER, head of the Division of

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Stratigraphy and Paleontology of the Illinois Geological Survey, has accepted a professorship in paleontologic geology at the University of Chicago, to fill the position made vacant when Dr. Carey Croneis became president of Beloit College. Dr. Weller will assume his new duties at the beginning of the autumn quarter.

DR. ELWYN L. PERRY has been appointed Edward Brust professor of geology and mineralogy at Williams College, and Dr. Elbert C. Cole has been appointed Samuel Fessenden Clarke professor of biology.

DR. LEWIS CLINE has been appointed associate professor of geology at the University of Wisconsin.

DR. HUDSON JOST, research associate in psychophysiology at the Mooseheart (Illinois) Laboratory for Child Research, of which Dr. Martin L. Reymert is director, has been appointed assistant professor in the department of neurology and psychiatry and director of the psychophysiological laboratory of the Medical School of the University of Tennessee at Memphis.

DR. JOHN C. KRANTZ, JR., professor of pharmacology in the School of Medicine of the University of Maryland, has received the sum of \$4,000 from the Ohio Chemical and Manufacturing Company to continue his studies in anesthesia.

DR. J. A. HALL, who has served as principal biochemist for the past three years for the U. S. Forest Service, Washington, D. C., has become director of the Pacific Northwest Forest Experiment Station at Portland, Ore.

DR. HAMILTON B. G. ROBINSON, of the Dental College of the Ohio State University, has been elected a member of the board of the American Cancer Society.

DR. HERBERT L. MASON, associate professor of botany of the University of California at Berkeley, has undertaken a five-year survey of the marsh flora of California. Under the auspices of the Fish and Game Commission, the project is financed by the Pittman-Robertson Act of Congress, whereby funds are given to the States by the Federal Government for certain types of research.

DR. L. B. ARNOLD, JR., recently assistant director of the division of chemistry of the Metallurgical Laboratory of the University of Chicago, has joined the staff of Arthur D. Little, Inc., Cambridge, Mass.

DR. E. C. WILLIAMS has resigned as vice-president and director of research of the General Aniline and Film Corporation, but will remain as consultant to the president in technical and development matters. Dr. William E. Hanford, manager of the central research

laboratory at Easton, Pa., has been named director of research of the company.

MRS. YVETTE HARDMAN EDMONSON, instructor in the biological sciences at Bennington College, has leave of absence to assist in a research program of the Woods Hole Oceanographic Institution on the chemistry and ecology of salt water ponds.

DR. WARREN ANDREW, associate professor of histology at Southwestern Medical College, has been invited to teach and carry on research for four months at the University of Montevideo, Uruguay. The Division of Cultural Cooperation of the U. S. Department of State has offered to finance the trip, which has been authorized by the Uruguayan Minister of Public Health.

DR. C. G. PHILLIPS, who is now serving with the rank of captain in the R.A.M.C., has been elected to an official fellowship as lecturer in physiology at Trinity College, Oxford.

A REUTERS dispatch reports that Charles H. Powell, president of the American Institute of Electrical Engineers and an official of the Westinghouse Electric and Manufacturing Company, will be chief of the electrical and radio branch of the United States group control council for Germany. He will establish controls under which the German industries will be permitted to resume or be converted to the manufacture of civilian goods. It will also be his task to see that the German electrical and radio producers do not manufacture war material or contribute to the production of armaments.

THE annual meeting of the Society for the Study of Development and Growth, which lasted for four days, was held under the presidency of Dr. B. H. Willier, professor of embryology at the Johns Hopkins University, at North Truro, Mass., beginning on July 24. The Lankenau Hospital Research Institute of Philadelphia and North Truro and the Marine Experimental Station for Cancer Research, of which Dr. Frederick S. Hammett is director and Dr. Theodore S. Hauschka associate director, were hosts at the meeting.

THE President and Fellows of Harvard University have received a bequest of \$50,000 to be held as a separate fund, the income from which is to be used for the upkeep of a chemical laboratory and for research and the study of chemistry.

THE Alcoa Mining Company has recently established a fellowship in the department of geology of Columbia University to be available for a graduate student in geology from Venezuela. It is the purpose of the fellowship to encourage young Venezuelan students in the study of lateritization.

A CONTRACT has been made between Union College and the American Locomotive Company, in which it is agreed that the company will enlarge the physics laboratories of the college in exchange for the use of a laboratory and other facilities and help on specific technical problems by members of the college faculty. The company has agreed to provide and supervise the building of ten or twelve new laboratories, and the facilities and faculty at the college are to be available to the company for five years. Faculty and research personnel will be available for at least four meetings a year. It has been arranged that representatives of the college and company will make shop inspection trips, followed by discussion of any company problems submitted.

A PROPOSAL is now before Governor Frank J. Lausche of the State of Ohio and members of the legislature for the erection of a State Health Center at the Ohio State University. An appropriation of five million dollars is requested to cover the cost of the center as the result of two years of planning and study by members of the medical and dental faculties and by representatives of these professions. The proposed center has been approved by official representatives of the medical colleges of Western Reserve University and the University of Cincinnati. It has also been approved by the Inter-University Council, which includes representatives of the six state universities—Bowling Green, Kent, Miami, Ohio, Ohio State and Wilberforce. Joining also in active support of the project are medical, dental and nursing

alumni of the Ohio State University, under the chairmanship of Dr. Russel G. Means, Columbus.

It is reported in the daily press that the will of Vincent Bendix, inventor and founder of the Bendix Aviation Corporation, who died on March 29, provides that 78 per cent. of the residue of his estate be left in trust for the establishment of the Bendix Foundation to assist in the teaching of science.

DR. JAMES GREENWOOD, JR., has given a fund to the Medical Branch at Galveston of the University of Texas for the establishment of the James Greenwood lectureship in neurology and neuro-surgery, in honor of his father. The lectureship will provide for meetings both at the Baylor Medical College in Houston and at the Medical Branch at Galveston of the university.

THROUGH an error, it was stated in SCIENCE that the *Biometrics Bulletin* was issued monthly. It appears bi-monthly. The first issue appeared in February of this year. It is the official organ of the Biometrics Section. The American Statistical Association has long published a journal and a bulletin which are concerned with the more general aspects of statistics. The new *Biometrics Bulletin* attempts to meet the specific needs of biologists who use statistical method in their work and of statisticians who work with biological data. Inquiries concerning the Bulletin should be addressed to the American Statistical Association, 1603 K Street, N.W., Washington 6, D. C.

SPECIAL ARTICLES

CONCERTED ANTIBIOTIC EFFECT OF PENICILLIN, METHIONINE, THREONINE AND METHIONINE SULFOXIDE UPON BRUCELLA, EBERTHELLA, SALMONELLA, AND SHIGELLA^{1,2,3}

PREVIOUSLY published^{4,5} and more recent unpublished observations demonstrated that broth, casein digest and blood serum antagonized the effect of penicillin upon certain Gram-negative microorganisms. The antagonism was at least in part due to the amino-acid contents, inasmuch as it could be also obtained with monoaminodicarboxylic acids, and pos-

sibly, histidine and arginine. No antagonistic effect was shown by hydroxyamino and diaminomono-carboxylic acids and methionine sulfoxide. Methionine was capable of reversing fully the antipenicillin effect of the monoaminodicarboxylic acids and only incompletely that of casein hydrolysate, broth and blood serum. However, upon addition of methionine in combination with threonine and methionine sulfoxide there appeared a marked enhancement of penicillin activity against highly refractory Gram-negative microorganisms in the presence of the antipenicillin factors as illustrated by the following experiments which were all carried out in meat inferior broth:

The measurements of the effect of penicillin were carried out in the manner previously described. The amount of bacterial growth was expressed in optical

¹ From the Division of Bacteriology, Laboratories of the Mount Sinai Hospital, New York, N. Y.

² The author wishes to acknowledge thankfully the accurate and capable assistance of Miss Alice Fisher.

³ The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for clinical investigations recommended by the Committee on Chemotherapeutics and Other Agents of the National Research Council.

⁴ Gregory Schwartzman, SCIENCE, 100: 477, 1944.

⁵ Idem, SCIENCE, 101: 276, 1945.

⁶ The methionine sulfoxide was generously supplied by Dr. Alfred Barol, director of the Department of Pharmacology of Wyeth Institute of Applied Biochemistry, at the recommendation of Dr. Paul György. SMACO dl-methionine and dl-threonine were used in these studies.

density.⁴ Solutions of amino acids in water were adjusted to pH 7.0-7.2 with sodium bicarbonate and sterilized by filtration through Berkefeld V candles. Forty-eight-hour-old cultures of *Brucella* and five-hour-old cultures of the remaining organisms served as inoculum. In all tests the initial number of organisms was 0.75×10^6 cells per ml. In repeated experiments methionine, 3.75 mg per ml, used alone and in mixture with threonine, 1.25 mg per ml, gave variable inhibition of the organisms studied (*i.e.*, 10 to 55 per cent.). The inhibitory effect of mixtures of methionine, threonine and methionine sulfoxide with and without penicillin is shown in Table 1.

TABLE 1
ENHANCEMENT OF PENICILLIN SUSCEPTIBILITY OF REFRACTORY GRAM-NEGATIVE ORGANISMS WITH THE AID OF METHIONINE, THREONINE AND METHIONINE SULFOXIDE

Microorganism	Per cent. inhibition in broth and M.T.S.*	Penicillin tests	
		Coefficient of resistance†	
		Broth	Broth and M.T.S.
<i>Eb. ty. Rawlings</i> . . .	gr. p.‡	250	125
" " H (Oxford) . . .	0§	375	100
" " TLM	16	500	75
" " O (Oxford) . . .	gr. p.	1500	75
" " Mt. S.	13	75	30
<i>S. Newport, 563</i> . . .	0	37.5	37.5
" <i>typhi murium</i> , var. Binns	50	37.5	18.75
" <i>typhi murium</i> , var. Aertrycke . . .	gr. p.	5000	1500
" <i>paratyphi A</i> , var. Durozzo	0	2500	500
" <i>paratyphi B</i>	0	625	50
<i>Sh. Flexner I</i>	50	4500	1500
" <i>Sonnei</i>	0	8000	2500
" <i>dysenteriae</i> (Shiga)	20	375	75
<i>Br. Melitensis</i>	40	100	25
" " 73	46	1250	100
" <i>Capri</i> (Oxford) . . .	50	3750	100

* M.T.S. = Methionine, 3.75 mg + Threonine, 1.25 mg + Methionine sulfoxide, 3.125 mg per ml.

† Ratio of the minimal amount of penicillin giving complete inhibition of the organism studied (initial concentration 0.75×10^6 cells per ml) to the minimal amount of penicillin required for complete inhibition of *Staphylococcus*, strain H, initial concentration 5×10^3 cells per ml.

‡ gr. p. = Better growth than in broth alone.

§ 0 = The same amount of growth as in broth alone.

As may be seen from Table 1, penicillin susceptibility of broth cultures of highly refractory Gram-negative organisms may be significantly enhanced in the presence of methionine, threonine and methionine sulfoxide. The amino acids alone produced variable degrees of inhibition of some organisms, and no inhibition or even some growth promotion of others. Apparently, these effects bore no relation to the enhancement of susceptibility to penicillin obtained in the presence of the acids.

In the following experiments the enhancing properties of the amino acids were studied quantitatively. *Eb. typhosa*, strain TLM selected for the studies, was completely inhibited in broth alone by 10 O.U. of

TABLE 2
SYNERGISTIC ANTIBIOTIC EFFECT OF PENICILLIN, METHIONINE, THREONINE AND METHIONINE SULFOXIDE

Amino acids in mg/ml of broth			O.U./ml of penicillin required for complete inhibition
Methionine	Threonine	Methionine sulfoxide	
0.3-3.75			> 2.5
....	1.25-2.5	> 2.5
....		0.3-6.25	> 2.5
0.3-0.94	1.25-2.5	3.125-6.25	> 2.5
1.875	1.25	3.125-6.25	> 2.5
1.875	2.5	3.125	> 2.5
1.875	2.5	6.25	> 2.5
3.0	0.125-2.5		> 2.5
3.0	0.3-3.125	> 2.5
3.0		6.25	> 2.5
3.0	0.125-0.625	3.125	> 2.5
3.0	1.25	3.125	> 2.5
3.0	2.5	0.3	> 2.5
3.0	2.5	0.625	> 2.5
3.0	2.5	3.125	1.5
3.0	2.5	6.25	0.5
3.75	1.25	3.125	1.5
3.75	2.5	6.25	0.1

0 = 0 = 40 per cent inhibition.

The organism used was *Eb. ty. TLM* in initial concentration of 0.75×10^6 cells per ml. When tested in broth alone 10 O.U. per ml of penicillin were required for complete inhibition.

penicillin per ml. Methionine, threonine and methionine sulfoxide were tested separately and in various combinations against 0.1-2.5 O.U. of penicillin per ml, *i.e.*, the highest concentration of penicillin being 4 times smaller than the minimal amount necessary for complete inhibition in the absence of the acids. As may be seen from Table 2, enhancement of susceptibility to penicillin results from synergistic rather than additive action of the amino acids. No enhancement was found upon addition of each of the amino acids separately and of mixture of methionine sulfoxide and threonine. The presence of methionine in sufficient concentration was essential for the enhancement. Methionine sulfoxide and threonine facilitated reciprocally the effect of methionine, the same degree of enhancement having been obtained with decreasing amounts of methionine, provided the concentration of threonine and methionine sulfoxide was correspondingly increased. It is of interest to note that using optimum concentrations of the acids the increase in susceptibility of the organism tested to penicillin was one hundred fold as compared to its sensitivity in broth alone, *i.e.*, 0.1 and 10 O.U. per ml, respectively.

Observations were also made at short-time intervals on the rate of growth and H-ion concentration of cultures of *Eb. typhosa*, strain TLM, in the presence and absence of the amino acids. The mixture of the amino acids in highest concentrations employed in these studies brought about some prolongation of generation time during several initial hours of the log phase. The H-ion concentration of the cultures varied from pH 6.8 to 7.1.

SUMMARY

Penicillin susceptibility of highly refractory Gram-

negative organisms may be greatly enhanced upon addition of methionine, threonine and methionine sulfoxide. The action of amino acids appears to be synergistic rather than additive. Methionine is essential for the enhancement. Threonine and methionine sulfoxide facilitate the effect of methionine following a reciprocal quantitative relationship.

GREGORY SHWARTZMAN

THE BENZYL ESTER OF PENICILLIN

DURING the past several years, various types of esters of penicillin have been made in this laboratory in a search for derivatives possessing greater stability, a depot action or effectiveness by mouth. Crude preparations of the methyl, ethyl, n-butyl and benzhydryl esters have been described by others,¹ but were reported to hold "no great promise." However, we have found that the benzyl ester promises to be extremely valuable because of its ease of preparation, enhanced stability, ready oral absorption and powerful chemotherapeutic action.

Our benzyl penicillin G has been obtained as a colorless, hard glassy solid which shows a sharp increase in fluidity at 50° C. The ester was prepared by treating free penicillin in an inert organic solvent with an excess of phenyl diazomethane, any unreacted penicillin being extracted with sodium bicarbonate solution. Evaporation of the solvent yielded a resinous product which was readily purified.

Benzyl penicillin is stable at temperatures above 100° C., in contrast to penicillin salts. The ester is also much more stable in alcoholic solvents than any known penicillin salt. It has a very low solubility in water, but is soluble in alcohol, ether, chloroform, ethyl acetate and in the polyethylene glycol type of polymers. It is approximately 2.5 per cent. soluble in sesame oil or propylene glycol.

In vitro, pure benzyl penicillin exhibits about one thirtieth as much bacteriostatic activity as pure sodium penicillin against broth cultures of *Staphylococcus aureus*. This was shown by serial dilution tests in which the bacteriostatic concentration was found to be 1 microgram per cc; under similar conditions, 0.03 microgram or 0.05 international unit of pure sodium penicillin sufficed. By the cup-plate method, benzyl penicillin is relatively much less potent and shows approximately one four-hundredth of the activity of sodium penicillin.

In vitro, a substantial proportion of the theoretical activity can be demonstrated after splitting the ester by incubating with an aqueous extract of rat kidney. Presumably this regeneration is caused by enzymatic cleavage since boiled kidney extract fails to liberate any activity. Rat serum is almost as effective as rat

kidney extract, guinea pig serum shows some activity, while human, horse, rabbit and dog sera are inactive. The cup-plate, serial dilution and the Warburg respirometer procedures have been used to measure the activity so liberated. The observed activity is approximately one half that to be expected theoretically from the penicillin content of the ester. The highest value observed was 54 per cent. (using the cup-plate method). Hickey² recently reports obtaining a maximum of 26 and 16 per cent. regeneration from methyl and ethyl penicillin, respectively, using alkaline hydrolysis.

Benzyl penicillin dissolved in a vegetable oil and injected subcutaneously or given by mouth protects mice against lethal doses of streptococci and pneumococci. Mice were inoculated intraperitoneally with 0.3 cc of a 1:100,000 dilution of an 18-hour broth

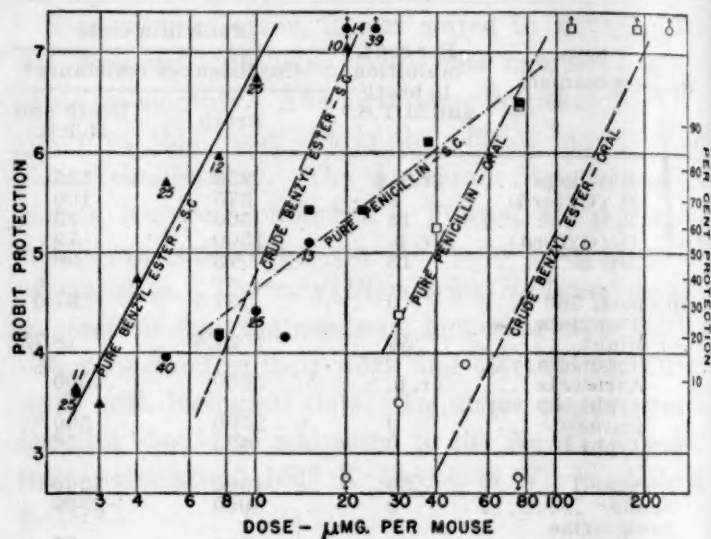


FIG. 1. Graph showing protection afforded by benzyl penicillin and pure penicillin against experimental streptococcal infection in mice.

Legend: ▲—Pure benzyl penicillin given subcutaneously, ●—Crude benzyl penicillin given subcutaneously, ■—Pure penicillin given subcutaneously, □—Pure penicillin given orally, ○—Crude benzyl penicillin given orally. Each point represents 15 mice unless otherwise indicated. The points to which arrows are attached represent survivals of either 0 per cent. (downward arrow) or 100 per cent. (upward arrow). In these experiments, 5 of the 63 inoculated control mice survived without any penicillin treatment.

culture of *Streptococcus hemolyticus* (Strain C 203). This inoculation kills 92 per cent. of the unmedicated control mice within 18 to 46 hours (average of 15 experiments). In the subcutaneous injection experiments 0.1 cc of a solution containing the desired dose of benzyl penicillin was injected one hour after the inoculation. For the oral experiments 0.1 cc was given by stomach tube one hour before and 4 hours after the inoculation. Observations were made at convenient intervals for the succeeding 72 hours; rarely were there any deaths after this time. The relative efficacies of 2 or more preparations were

² R. J. Hickey, *SCIENCE*, 101: 462-463, 1945.

¹ K. Mayer, G. L. Hobby and E. Chaffee, *SCIENCE*, 97: 205-206, 1943.

determined by comparing the mortalities observed following graded doses given to comparable groups of mice.

Fig. 1 presents the composite data of *in vivo* experiments in which graded doses were given of both crude and pure benzyl penicillin preparations dissolved in sesame oil and of a crystalline water-soluble salt of penicillin (1,600 μ /mg) suspended in sesame oil. From this log-probit graph³ the relative potencies of the 3 preparations as well as the efficiency of the two routes of administration may be estimated. In some of these tests the sodium penicillin was dissolved in an aqueous buffer solution, and it was observed that injections of sodium penicillin suspended in oil afford the same protection as the same amounts injected in buffer. When injected subcutaneously, the pure ester appears at least 3 times as potent as the pure salt on a weight basis, although some reservations must be made for a lack of parallelism in the straight lines relating dose to the degree of protection. If allowance is made for the difference in molecular weights the ratio becomes still greater. In other results not shown in the figure, the pure benzyl ester has been found to be 7.5 times as potent as an equimolecular amount of commercial sodium penicillin.

Of great interest also is the effectiveness of benzyl penicillin by mouth. In mice, as may be seen from Fig. 1, about 10 times as much of the crude benzyl penicillin (50 per cent. pure) was required orally as subcutaneously. A less pure preparation (10 per cent.) was better utilized by mouth, since only 4 times as much of it was needed for protection equivalent to that obtained from subcutaneous injections. The mouse appears to absorb water-soluble penicillin quite efficiently, since only three times as much (of either the pure or commercial grade salt) is required by mouth as subcutaneously. It should be noted that the mouse is a poor subject for oral administration experiments because of the relatively rapid passage of the drug through its intestinal tract. This situation favors the quickly absorbed water-soluble penicillin, since there is less exposure to adverse conditions in the stomach, but diminishes the action of the ester, which appears to require digestive cleavage before absorption.

The data presented above indicate that when injected subcutaneously in mice, benzyl penicillin is at least 3 times as potent as ordinary sodium penicillin in aqueous solution or suspended in oil. When taken by mouth, the benzyl penicillin is less active than by injection, but still is sufficiently potent to make it substantially as effective as an equivalent weight of sodium penicillin given by subcutaneous injection. The potential advantages of these favorable properties are obvious. Clinical data are being published

elsewhere⁴ which demonstrate that these advantages may also be seen in patients.

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THE FUNCTIONAL PATHOLOGY OF FROST-BITE AND THE PREVENTION OF GANGRENE IN EXPERIMENTAL ANIMALS AND HUMANS¹

THE functional pathology of frostbite has thus far been obscure. Apart from the excellent studies of Greene,² who has approached this problem mainly from the morphologic viewpoint, few basic facts were available. The use of the fluorescein test^{3, 4} has thrown more light on the pathologic physiology of the frostbite lesion. In this test small amounts of fluorescein are injected and its migration with the blood stream and into the interstitial spaces is observed under ultra-violet light.

Six rabbits were depilated on the abdomen and exposed to cold by applying the bottom of small glass beakers filled with dry ice to the skin. The periods of exposure varied from 5 to 90 minutes. Under this exposure the skin freezes solid and thaws after intervals varying between 5 and 25 minutes, depending on the length of exposure. For periods of 30 to 120 minutes following such refrigeration no fluorescein appears in the exposed areas indicating a severe spasm of the arterioles. After this time a second stage is initiated during which all blood vessels reopen and fluorescein can be seen throughout the exposed area. The diffusion of fluorescein into the surrounding tissues in the second stage is many times greater than in the non-exposed skin giving the picture of intense hyperfluorescence in the previously frozen areas. This period is also characterized by marked swelling of the exposed areas. Eight to fourteen hours after exposure a repeat fluorescein injection shows that now the exposed spots are not fluorescent, indicating a pre-gangrenous state. This non-fluorescence increases in the next hours until finally the entire spot is non-fluorescent and becomes gangrenous. Biopsies taken at this time show that, in agreement with the findings of Greene² and Krey-

⁴ T. O. Gamble, L. C. Miller and M. L. Tainter, *Am. Jour. Obst. and Gynec.* (in press).

¹ Aided by grants from the John and Mary R. Markle Foundation and the Council on Pharmacy and Therapeutics of the American Medical Association.

² R. Greene, *Jour. Path. and Bact.*, 55: 259-267, 1943.
³ K. Lange and L. J. Boyd, *M. Clin. N. A.*, 26: 934-952, 1942.

⁴ *Idem*, *Arch. Int. Med.*, 74: 175-184, 1944.

³ L. C. Miller and M. L. Tainter, *Proc. Soc. Exp. Biol. and Med.*, 57: 261-264, 1944.

berg,⁵ there is a clumping of red cells in the smaller vessels which is probably due to loss of plasma through the highly permeable vascular wall. The red cells are stranded and silt the blood vessels forming a sludge. They do not, however, represent true thrombi in the beginning. A simple injection of saline enables one to wash out these erythrocytes as individual cells. Only after approximately 72 hours does organization of these cells into thrombi occur. This thrombosis ultimately leads to gangrene.

It appeared obvious therefore that therapeutic attempts to avoid gangrene after frostbite must be started before the stage of thrombosis is reached.

Ten rabbits of equal weight were exposed to chilling by the method already described. Five of the animals were treated by heparinization within four hours after exposure.⁶ None of the heparinized animals developed gangrene, while in the untreated controls all areas exposed for more than 15 minutes became gangrenous. Encouraged by this result, the following experiment was done on 22 rabbits. One hind leg was exposed to an alcohol dry ice bath of -12° – 20° C. for a period of 45 to 90 minutes with the leg protected by a thin boot of condom rubber. After the exposure, eleven animals were heparinized, while eleven remained untreated. Of the treated animals only two showed some slight surface lesions, while the legs of the others remained completely intact with no gangrene. All controls lost their legs by complete gangrene, including the bone.

The practical demonstration of the therapeutic value of heparinization in the prevention of gangrene was made possible by the study of artificial frostbite in human volunteers. These volunteers were recruited from patients who were being treated for subacute bacterial endocarditis at the Jewish Hospital of Brooklyn by the combination of penicillin and heparin.^{7, 8} In one group the frostbite was accomplished by means of a porcelain crucible filled with dry ice and applied to the skin of the lateral aspect of the upper arm without pressure for ten minutes. An area of about 2 cm came in contact with the skin. By this method, a temperature of minus 22° C. was achieved. Heparinization was started immediately following exposure. One volunteer served as a control. The other group was subjected to local refrigeration in the same manner but for two exposures of 30 minutes each. The initial or control exposure was permitted to develop for six days before the second frostbite was induced, immediately following

which treatment with the subcutaneous heparin in the Pitkin menstruum was initiated.^{9, 10} The 30-minute exposure with dry ice results in temperatures considerably below minus 22 degrees Centigrade, and is comparable to the frostbite suffered by aviators in high altitude flying such as nose gunners after demolition of the plexi-glass protection or gunners attempting to un-jam guns without glove protection.

The clotting time in the treated cases stayed between 25 to 60 minutes. It was apparent from the observations in these human volunteers that all the adequately treated lesions escaped any deeper injury. One must recognize that these investigations in human volunteers are merely transition experiments which serve as added proof of the genesis of gangrene following frostbite and the validity of the therapeutic approach.

The further practical demonstration of the method's value was made possible when a frostbite case appeared at the Research Unit of the New York Medical College. A man was sent to the hospital following exposure to a temperature of 18 to 20 degrees F. for at least 14 hours while lying in the street. His hands were completely unprotected, while his feet were protected only by low shoes and thin socks. On admission his feet were ice-cold up to the knee and remained so for five hours after admission. He was heparinized by the intravenous route for five days, the clotting time being maintained between 30 and 60 minutes. He developed considerable blistering, especially on the hands, but completely escaped any permanent tissue loss. From the experience with similar exposures, one can say that this man without heparinization would probably have had more or less extensive loss of the extremities.

Experiments are under way to determine the simplest method of heparinization and the longest interval between exposure and start of therapy which would still be effective.

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ACTION SPECTRUM FOR THE PHOTO- PERIODIC CONTROL OF FLORAL INITIATION IN BILOXI SOYBEAN

THE effectiveness of light applied to Biloxi soybean leaves during the middle of the dark period to prevent

⁹ L. Loewe, P. Rosenblatt and J. Lederer, *Proc. Soc. Exp. Biol. and Med.*, 50: 53, 1942.

¹⁰ L. Loewe and P. Rosenblatt, *Am. Jour. Med. Sci.*, 208: 54–63, 1944.

⁵ L. Kreyberg and L. Rotnes, *Acta. Path. Microb. Scand.*, 11: 162, 1932.

⁶ K. Lange and L. J. Boyd, *S. G. and O.*, 80: 346–350, 1945.

⁷ L. Loewe, P. Rosenblatt, H. J. Greene and M. Russell, *Jour. Am. Med. Assn.*, 124: 144–149, 1944.

⁸ L. Loewe, *Bul. N. Y. Acad. Med.*, 21: 59–86, 1945.

turned to the 16-hour photoperiod. Floral initiation was determined by dissection eight days later.

Results of an experiment covering the region from 5,700 A. to 6,900 A. are shown in Fig. 1. Cross lines on the graph indicate the ten variations of energy used in the experiment. The figures on these lines are the numbers of flower primordia produced on four plants and their positions along the lines correspond to the wave-length at the middle of the station where they were irradiated. In this experiment there was a gradation of effect with decreasing energy at a specific wave-length. Results of an experiment in the green to violet region of the spectrum are shown in Fig. 2. The efficiency of radiation for interrupt-

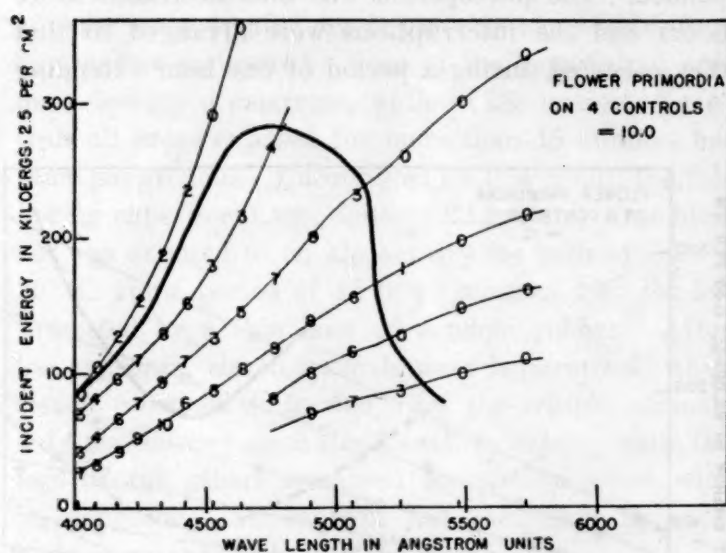


FIG. 2. Action spectrum in the green-violet region.

ing the dark period in this experiment decreases from the red to 4,600 A. About 800 kiloergs per cm^2 are required to prevent formation of flower primordia in this region, while 30 to 50 kiloergs per cm^2 are sufficient in the red. Below 4,600 A. effectiveness increases and another maximum is reached near 4,000 A.

Results for many experiments have been combined in a curve, covering the region from 3,800 A. to 7,200 A. (Fig. 3). The region from 7,200 to 20,000 A. has also been investigated, but floral initiation was not inhibited beyond 7,200 A. Floral initiation can be suppressed by interruption of the dark period with light of sufficient energy from any region of the visible spectrum, but there are two regions of maximum efficiency, one in the yellow, orange and red and the other in the violet near 4,000 A. The response curve was very accurately determined from 4,000 A. to 5,100 A. and from 5,500 A. to 7,200 A. and the incident energy required to prevent floral initiation is accurate within each region, but difficulty was encountered in connecting the two. The energies indicated for the blue-violet are uncertain by a possible twofold factor relative to those in the red. This was a result of using different lots of plants for the two

regions and of the necessity for irradiating longer times in the blue-violet than in the red and over periods removed by as much as 30 minutes from the middle of the dark period.

The over-all response curve has striking similarities to the curve for photosynthetic utilization of carbon dioxide. In particular it shows the same action limit in the red and two maxima, one in the red and the other in the blue. The curve indicates that the chloroplast pigments of the leaf are associated with the dark period interruption reaction. Carotenoids apparently are not involved in the light absorption re-

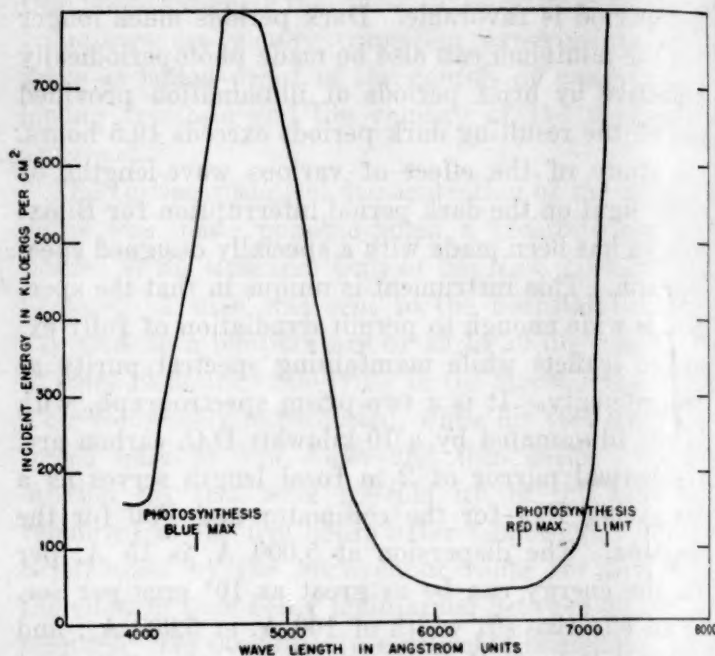


FIG. 3. Composite action spectrum based on 21 experiments. Any point on the curve shows the amount of energy that must be applied to a Biloxi soybean leaflet during the middle of the dark period to prevent flower initiation. The points of maximum utilization of CO_2 in photosynthesis and the limits of this reaction, as shown by Hoover,¹ are included for reference.

sponsible for the reaction, since their maximum absorption is in the range of minimum effectiveness and they are transparent in the red. It is likely that the action spectrum is due to a porphyrin-like material which is probably chlorophyll. The action spectrum is not identical with that for carbon dioxide utilization, as might be anticipated, but the significance of the differences between the two require detailed analysis.

A possible explanation for these observations is that energy absorbed by the chlorophyll is transferred to a reaction leading to the destruction of a material determining floral initiation. This may be a photo-oxidation, even though the photo-response was found to be independent of oxygen pressure.

The action spectrum is being determined for *Xanthium pensylvanicum*, and its response in the red

¹ W. H. Hoover, Smithsonian Miscellaneous Collection, Vol. 95, No. 21, 1937.

has been found to be similar to that of Biloxi soybean.

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A NEW POLYSACCHARIDE FROM BLACK SPRUCE (*PICEA MARIANA*)

WHEN native lignin is isolated from black spruce (*Picea mariana*) with aqueous alcohol and the alcohol is distilled off under reduced pressure, a mixture of native lignin and resins separates from the remaining aqueous solution.¹ On saturating the aqueous filtrate with sodium sulfate, a polysaccharide separates as a flocculent precipitate which, after centrifuging and washing with 80 per cent. alcohol, then with absolute alcohol, and finally with ether and petroleum ether, is obtained as a light powder. After purification by prolonged electrodialysis and repeated precipitations by dropping a concentrated aqueous solution into absolute methanol, the polysaccharide is obtained as a white, nonhygroscopic powder in a yield of about 0.1-0.2 per cent. of the wood. It does not reduce Fehling solution before hydrolysis with hot dilute hydrochloric acid, but does so very strongly after this treatment. In spite of lengthy electrodialysis, it still contains 0.7 per cent. ash (determined as sulfate). It is very soluble in water, forming a slightly turbid solution similar to starch solutions. Its aqueous solution shows a slight levorotation which, after hydrolysis, changes to a strong dextrorotation. A hydrolysis curve, obtained by boiling the polysaccharide with 2 per cent. sulfuric acid, shows that a maximum

reducing power of about 95 per cent. sugar (calculated as glucose) is reached after 6 hours. The presence of 0.7 per cent. MeO and a slight residue left after hydrolysis indicate that a small amount of lignin is still present which is difficult to remove because of the colloidal properties of the polysaccharide. On distillation with 12 per cent. hydrochloric acid, the polysaccharide gives 3.3 per cent. carbon dioxide, which corresponds to 13.2 per cent. uronic acid. When the polysaccharide is acetylated by heating it with a mixture of pyridine and acetic anhydride, a gelatinous suspension is formed from which an acetylated product is obtained which is insoluble in water and the common organic solvents.

A biochemical analysis² of the hydrolyzed polysaccharide by the method of Wise and Appling³ shows the presence of 72.6 per cent. galactose, corresponding to 65.3 per cent. galactan. The polysaccharide also contains 13.1 per cent. arabinose (determined by the method of Wise and Peterson⁴) corresponding to 11.5 per cent. araban; glucose, mannose and xylose are absent. The presence of uronic acid, the levorotation and the insolubility of the acetylated derivative differentiate the polysaccharide from the arabogalactans isolated from certain larch species.^{5,6} As the acetate of arabogalactan is soluble in organic solvents,⁵ it is improbable that the polysaccharide is a mixture of arabogalactan and polyuronic acid because, in this case, the acetate should be at least partially soluble in organic solvents.

From the above analysis, it seems that the 3 components—galactose, arabinose and uronic acid—are present in the polysaccharide in a 4:1:1 molecular ratio.

A closer chemical investigation of this polysaccharide is in progress and the results will be reported at an early date.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE WATER MANOMETER FOR RECORDING INTESTINAL ACTIVITY¹

EXPERIMENTS on intestinal activity *in situ* often fail because of unsatisfactory recording equipment. This tends to discourage performance of such experiments in student laboratories. This note describes a simple device for recording intestinal motility which has the advantage of economy, ease of construction and maintenance, and which may be readily adjusted to give a wide range of initial force distending the

intestine. Furthermore, by the use of a lever, considerable amplification of changes in the manometer level may be obtained readily.

² The author is indebted to Dr. P. Cundy, of the Analytical Department and Mr. J. F. McCoy, of the Bacteriological Department of the Institute, for carrying out the analyses.

³ L. E. Wise and J. W. Appling, *Ind. Eng. Chem., Anal. Ed.*, 16: 28, 1944; 17: 182, 1945.

⁴ L. E. Wise and F. C. Peterson, *Ind. Eng. Chem.*, 22: 362, 1930.

⁵ F. C. Peterson, A. J. Barry, H. Ukauf and L. E. Wise, *Am. Chem. Soc.*, 62: 2361, 1940; and preceding papers.

⁶ E. V. White, *Am. Chem. Soc.*, 64: 2838, 1942; and preceding papers.

¹ F. E. Brauns, *Am. Chem. Soc.*, 61: 2120, 1939.

² Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

As may be seen from the diagram (Fig. 1), the device is composed of parts already available in the laboratory. It is essentially a water manometer, the entire system from intestinal balloon to manometer float being filled with water. The manometer consists of an inverted Kimble retempered glass culture tube (No. 45070), 15 by 150 mm (or similar tube of uniform bore with or without the side arm indicated). This size has been found suitable for use in cats. In

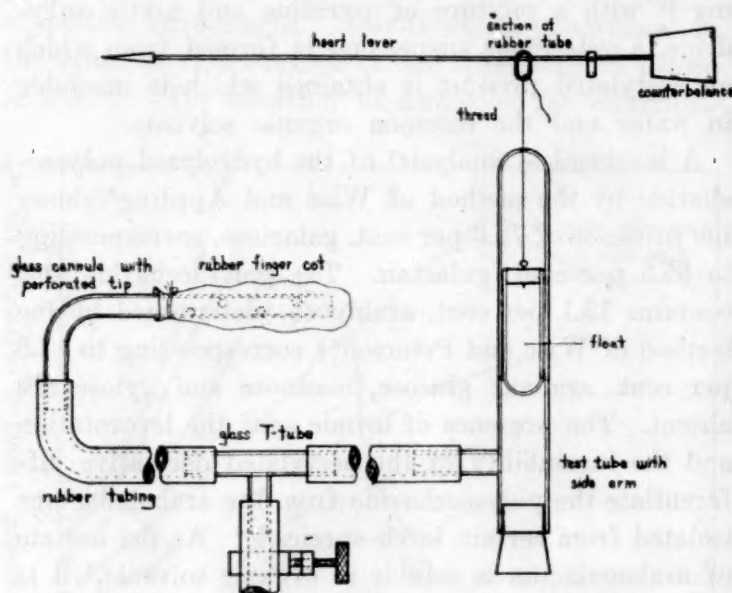


FIG. 1.

the bottom of the tube a pin hole is blown centrally through which a silk thread may pass freely. A manometer float is made either of a hypodermic syringe piston or of a Kahn tube, the size being selected by trial and error to fit so snugly that only a thin film of water separates the float from the manometer wall. Such close fitting insures accurate float reproduction of water level changes. The top of the float is closed with a rubber stopper about 5 mm thick to the center of which is attached a silk thread. The other end of the thread, after passing through the hole in the inverted tube, pierces the wall of a narrow section of rubber tubing which is also pierced by the writing arm of a Harvard heart lever. This latter arrangement makes possible quick adjustment of amplification of the water column height without the necessity of moving the lever relative to the recording surface, and without the use of clamps on the lever or knots in the thread. The recording lever is suitably counterweighted with a small rubber stopper. The intestinal balloon is of the conventional finger-cot variety, throughout the length of which runs a glass tube with several perforations. The perforations allow collapse of the balloon on one side or the end of the tube without interfering with transmission of pressure changes to the manometer. The system is preliminarily filled with water and after the balloon

has been placed *in situ* the level of the float is adjusted by a syringe via the side arm of the T-tube.

We have found this device very easy to maintain and to adjust for operation. It is obvious that alignment of the manometer with the recording surface is not critical, and with the use of the gravity-type heart-lever little or no attention is required during the course of an experiment. With the dimensions described, excursion of several centimeters of the writing point per cc volume change is easily achieved. The inherent frictional inertia of the manometer is not a serious drawback to its use for the relatively slow changes in activity seen in the stomach and intestine. The device should prove useful for recording changes in volume of kidney and spleen.

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USE OF A DOUBLE-NOZZLED SPRAY APPARATUS FOR THE APPLICATION OF DDT OR OILS

DURING studies of methods of applying DDT (1-trichloro-2,2-bis(p-chlorophenyl)ethane), a deposit superior in quantity and quality was obtained by combining the water and DDT solution after they leave the spray nozzle. This was done by combining a spray of each from a pair of nozzles converging at an angle of approximately 35 degrees. A DDT solution was sprayed from a small atomizer into a water spray coming from a suction type paint sprayer. The droplets of the two sprays mixed by collision about 2 to 4 cm away from the nozzles.

When the DDT solutions were prepared with water-miscible solvents such as acetone, dioxane or alcohol, the insecticide was deposited as "nascent precipitates," some liquid and some solid. The DDT was precipitated when the droplets of the organic solution mixed with the droplets of water, and was immediately deposited on the sprayed surface.

Deposits on glass were found to consist of small particles well distributed over the surface, and it was possible to vary the type of the particle by varying the DDT concentration or the solvent. Oranges and apples were also successfully sprayed by this method.

Solutions of DDT in solvents other than those miscible with water were also successfully dispersed in this apparatus. Microscopic examination of the sprayed droplets showed that liquids, such as mineral oil, were dispersed in the water phase very much as if the oil had been emulsified, but in such an unstable condition that the oil deposited on the glass or fruit surface, leaving the water to drain off. The instability of the oil-in-water dispersion makes it possible to build a heavy deposit of oil on the surface

without losing much of it in the water that is draining away.

The deposits of DDT on glass were extremely resistant to water applied as drops similar to rainfall. The effect on the surface of an apple was more difficult to observe precisely, but there was no evidence that the DDT washed off the apples any more readily than from the glass. Some nascent precipitate of DDT from acetone spray on glass was not visibly decreased after 7 hours of pounding from rapidly dropping water.

Some of the advantages of using a multispray process over the spraying of a concentrated DDT solution alone may be revealed after the practical trials. At present it appears that inclusion of the water spray in the process should be useful as a carrying medium in orchard or truck-crop spraying. The presence of water should also decrease the danger of injury to foliage.

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MARKING ANOPHELES MOSQUITOES WITH FLUORESCENT COMPOUNDS¹

MANY investigations concerned with activities and life cycles of *Anopheles* mosquitoes, as well as other insects, necessitates marking specimens in such a manner that individuals so marked can be recognized when subsequently collected and examined. Methods previously used employed dyes, either in solution or in the form of small particles, and metallic dusts.² When these methods are used, individual specimens have to be handled when search of collections is made for marked specimens. This procedure is very time-consuming.

The method herein presented involves the use of fluorescent compounds for marking adult specimens of *Anopheles quadrimaculatus* Say and later detecting those marked under an ultra-violet light. Anthracene, rhodamine B and fluorescein which produce blue, red and green fluorescent colors, respectively, have been successfully used as outlined below.

Anthracene can be applied as an aerosol or as a dust mixed with gum arabic. The aerosol is made by vaporizing anthracene with heat into a closed chamber. Particles with a mean diameter of 6.7 microns are produced. Exposure of caged specimens for five minutes to an aerosol concentration of 10.0 milligrams per liter of air produces an homogenous deposit of particles on the exoskeleton. This treatment apparently does not harm the specimens in any way.

When used as a dust, anthracene is mixed with gum arabic in water in the ratio of 1 part anthracene to 2 parts gum arabic. The mixture is evaporated to dryness and ground to a powder. Specimens are dusted with the powder and then placed in an atmosphere of saturated humidity for 15 minutes. This causes the particles to deliquesce and adhere to the insects. The use of gum arabic as a diluent provides a firm adhesion, thus contamination of unmarked specimens in the process of collection is avoided.

Rhodamine B and water-soluble fluorescein can be used to dye gum arabic at a concentration of 10.0 milligrams of dye to 3.0 grams of gum arabic. The resulting mixture is used as the anthracene dust for marking specimens, as indicated above.

By the use of this method large numbers of individuals can be readily marked and the examination of several hundred specimens can be made in a matter of a few minutes. Further details of this method will be given in a later publication.

JOHN W. ZUKEL

U. S. PUBLIC HEALTH SERVICE

DISCUSSION

ANTIBACTERIAL ACTION OF QUINONES

WITH reference to the introductory statement by Colwell and McCall in their article in *SCIENCE* for June 8, page 592, that "the antibacterial activity of quinones has been recognized since 1911," I wish to remark that this property of quinones was made use of by me for preventing the bacterial decomposition of sugar-cane juice in 1906, in a study upon the action of the oxidases of cane juice on various polyphenols. I quote as follows from my article on "The Fermentation of Sugar-Cane Products":¹

¹ From Emory University Field Station, Newton, Georgia.

² D. E. Eyles, *Public Health Bulletin*, No. 287, 39 pp., 1944.

If certain polyphenols, such as hydroquinone, or pyrogallol, are added to fresh cane juice, a rapid oxidation of these compounds is produced with an intense darkening of the juice. The latter takes on at the same time a peculiar odor, due to the formation of a quinone body, and what is more remarkable acquires a germicidal property which, in the case of the juice treated with hydroquinone, insures its preservation for weeks. Sterilized juice shows no change in color and develops no germicidal properties with any of the phenol bodies named. In connection with this oxidation of hydroquinone there is a very marked absorption of oxygen.

Other experiments in my article tended to show that the familiar darkening in color of expressed

¹ *Jour. Amer. Chem. Soc.*, April, 1906, pp. 455-6.

cane juice and its incipient slight germicidal action are due to the action of the oxidases of the juice upon naturally occurring polyphenols of a tannin nature with production of quinone-like substances. The discoloration is more evident when freshly cut sections of the sugar-cane are exposed to the air and this leads to the suggestion that this reaction may serve a useful purpose in protecting living plants to a certain extent against the invasion of micro-organisms when their inner tissues are exposed to the air as a result of injury. The antibacterial activity of quinones was very likely known before the year 1906, but I am unable at the moment to refer to previous observations recorded in the literature.

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GREEN COLOR OF PLANT ASH DUE TO MANGANESE, NOT TO COBALT

IN 1932, Bishop and Lawrenz¹ published a note on the presence of cobalt in plant ash. Their attention was called to the problem by the color of the ash which was white from some plants but of various shades of green from others. Iron, chromium, manganese, cobalt, nickel and copper were considered as possible causes of this green color. Using the magneto-optical method of analysis it was concluded that cobalt was responsible for the green color of the ash. This has been widely quoted as evidence of cobalt in plants.²

In general plants contain but a fraction of a part per million (dry weight basis) of cobalt. The manganese content, however, is commonly over fifty parts per million and may reach as high as 4,300 parts per million manganese in tree leaves growing on acid soil.

From the evidence adduced from the color and composition of the ash from several thousand of plant ash analyses, and from the color of ignited soil extract residues we can state quite positively that the cause of the green color of plant ash is due to manganese. However, to develop this color it is necessary that the ash be thoroughly oxidized, heated at a fairly high temperature and that the ash be relatively high in potassium (or sodium) carbonate. Under these conditions green potassium manganate is formed. The green color developed is the classical and time-honored qualitative test for manganese by fusion with sodium or potassium carbonate under oxidizing conditions. It is a very sensitive qualitative test. Plant ash frequently contains enough manganese to develop the colors, when treated with water, which are characteristic of chameleon mineral.

¹ SCIENCE, 75: 1940, 264-5.

² L. G. Willis, "Bibliography of the Minor Elements," third edition, p. 281, 1939.

If green plant ash is leached with water, a green solution is obtained. This solution turns pink when neutralized with hydrochloric acid, as Bishop and Lawrenz state. This is characteristic of manganese and not of cobalt. The cobalt of plants is far too low to yield these colors. Further, it is doubtful if any cobalt would go into solution in a water extract of plant ash because cobalt is not dissolved by a slight excess of alkali except under unusual conditions such as in the presence of ammonia. The large excess of phosphate in plant ash would also tend to prevent the cobalt from dissolving in water.

The addition of quantities of cobalt up to five parts per million, the highest quantity plants seem to contain, has no noticeable effect on the color except in the case of plants high in aluminum such as the sweet leaf. In this case the change in color is the development of a faint shade of blue rather than green.

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THE COLOR REACTION OF VITAMIN A ON ACID EARTHS

A BRIGHT blue color reaction of vitamin A upon adsorption on a commercial adsorbent made from Montmorillonite was lately described by Lowman¹ and, in a recent issue of this journal, Zechmeister and Sandoval² point out that a similar observation had been previously reported by Meunier.³ However, this observation goes further back; in 1939 Emmerie and Engel⁴ discovered that vitamin A gives a dark blue color, and carotenoids a bluish green color, when adsorbed on Floridin SX used for the removal of vitamin A and carotenoids from serum extracts prior to the reductometric colorimetry of tocopherol. When adapting the method of Emmerie and Engel to a photoelectric procedure, we confirmed their observation and described⁵ that a clear yellow benzene solution containing, besides tocopherol, vitamin A and carotenoids caused a column of floridin earth to assume a dark greenish blue color.

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¹ A. Lowman, SCIENCE, 101: 183, 1945.

² L. Zechmeister and A. Sandoval, *ibid.*, 101: 585, 1945.

³ P. Meunier, *Comptes rendus de l'Acad. Franc.*, 215: 470, 1942.

⁴ A. Emmerie and C. Engel, *Rec. trav. chim. Pays-Bas*, 58: 283, 1939.

⁵ G. Gernsheim Mayer and H. Sobotka, *Jour. Biol. Chem.*, 143: 695, 1942.

CORRECTION ON "CHRONIC INTERMITTENT ANOXIA . . ."

THE writer is embarrassed by his discovery that in revising a manuscript entitled "Chronic Intermittent Anoxia and Impairment of Peripheral Vision," an important paragraph was inadvertently omitted from the version which appeared in *SCIENCE* for June 15, 1945. In checking back it becomes evident that the omission arose in the author's final copy, and that the editors of *SCIENCE* are in no way responsible. The paragraph which should have been inserted between the two paragraphs of column 2, page 615, is as follows:

Serial determinations of alveolar gas tensions for each visual test day were carried out on sixteen of the twenty subjects by Dr. Wright Adams, but no relationship could be established with the impairment of peripheral vision. Similarly, in a smaller number of subjects no clear relationship could be established between the visual effect and renal vascular changes (Dr. Alf S. Alving), cardio-vascular changes (Dr. Emmet B. Bay), blood chemistry (Dr. Guzmán E. S. Barrón), psychiatric changes (Dr. Hugh T. Carmichael), electroencephalograms (Dr. Theodore Case), peripheral blood flow (Dr. Milton Landowne), certain metabolic effects (Drs. Henry T. Ricketts and A. Hughes Bryan), and certain additional neuropsychological indicators.

Through omission of the above paragraph, inadequate representation was given to the scope of the total war research project in which the psychological studies carried out by the writer constituted one aspect. In addition to their particular lines of investigation, each of the above men contributed generously of specialized knowledge and of time and energy to insure adequate clinical supervision of the experimental subjects and a valid interpretation of the impairment of peripheral vision as reported.

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HOW STENTOR ANCHORS ITSELF

FOR two hundred years it has been known that the large ciliated infusorian called stentor alternates between free-swimming and fixed or temporarily anchored conditions. Swimming free it may find localities suitable for itself and its progeny; but temporarily fixed it feeds itself, joins with a neighbor and procreates its kind. Though the fixed state is so important our knowledge is far from satisfactory as to the way in which the animal anchors itself so readily and so readily breaks away again.

The early microscopists observed a slight enlargement at the foot end of the body and considered this functioned as a sort of sucker to hold the animal fast. And as late as 1926 precise methods of sectioning

stentor revealed what was interpreted as a mechanism to hold the animal fast by aid of atmospheric pressure.

However, the general understanding is that stentor holds fast by the aid of pseudopodia. These are of two sorts, large branches of the foot and fine threads that have been called setae-like cilia and also pseudocilia. But it has been contended that the anchorage is chiefly by cilia that form a brush or "scopula" as found in some other ciliates. There is also the view that upon certain substrates stentor anchors itself merely by the stickiness of the exposed cortex, a subectoplasmic layer that comes to the surface at the middle of the foot.

Prolonged observation of *Stentor coeruleus* leads me to reject any sucker hypothesis but enables me to combine the other views as follows:

Stentor anchors in a series of actions that may or may not go on to the most complex expression. The foot as an abruptly truncated ending of a stalk is applied to a suitable substrate and the central cortex adheres and spreads out. Some of the cilia of the lower end of the body show remarkable activity till they reach and stick to the substrate, whereupon they transform into the stiff radiating fine pseudopodia or pseudocilia that hold the animal firmly. Finally the entire foot region flows out radially as gross pseudopodia, that do but extend the area of activity of the real adherents, the cortex and cilia. The actual adherence is by the stickiness of the cortex and of such cilia as transform into holdfasts. Thus the large pseudopodial branches of the foot, so evident in many illustrations of stentor, are important fixation organs in the sense that they increase efficiency by spreading the adhering surfaces of the cortex and cilia over a larger area.

In the swimming phase the animal has a minimum exposure of cortex at the middle of the foot and this is surrounded by cilia. These two elements evolve and also the whole foot with its colored and ciliated stripes spreads out radially as the ectoplasmic component of the completed foot disk. This disk is variously adjusted to fit against different surfaces, flat or convex or linear. Its outlines slowly change like those of an amoeba.

When the body contracts from external stimuli, or inner states, the foot disk also contracts. Either known stimuli or unknown inner changes may lead to the breaking away of the disk. This is commonly instantaneous but may show stages in which there is dedifferentiation of the attaching organs ending in the gross pseudopodia being withdrawn into the foot and the finer pseudopodia changed back into locomotor cilia of the body.

Polarity in stentor then is marked by great diversities in structure and action at the two poles. The

peristome is highly specialized for the collection, selection and ingestion of food by means of ciliary and myonemal structures. But at the opposite pole the foot frequently differentiates and dedifferentiates anchoring organs. These are made by the sticky cortex and the brush or "scopula" of cilia that change function, but there is also a reversion to primitive rhizopod activity involving all parts of the foot.

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THE THREAT OF ANTI-VIVISECTION

SOME careful consideration by appropriate scientific bodies should be given to the threat of anti-vivisection which has flared up recently, particularly in New York State.

The Hearst press succeeded in inflaming the uninformed layman against animal experimentation and medical research. Medical opposition was weak and very nearly ineffectual. If more concrete measures can not be adopted soon it is not impossible that state and even national legislation will be enacted to end scientific animal experimentation.

The means for handling pernicious legislation of this kind exists, but laxity in employing it has created the necessity of calling this fact to the attention of those who will realize its import.

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THE MATHER COLLECTION OF PORTRAITS

THE National Park Service has placed in the Library of Congress the nucleus of a collection of portraits of national park executives as a memorial to Stephen Tyng Mather, first director of the National Park Service. This collection marks a new approach to the history of national parks by bringing together portraits of the most prominent persons who have been associated with national park work and is intended to include portraits of at least 500 individuals. The collection will include (1) Park executives, present and past; (2) persons who have been associated with the establishment and development of national parks and national monuments; (3) persons who have contributed to national park art and literature, and (4) persons whose names are associated with these areas either through place names or designations of species which occur in these areas.

The collection is filed in the Division of Prints and Photographs in the Library of Congress in vertical steel cases. Each portrait is placed in a standard size envelope marked with the full name and year of birth of the individual and is accompanied by an index card giving at least six items of information, viz.,

(a) full name; (b) date and place of birth; (c) position; (d) titles of the author's publications, if any; (e) reference to a published biography, if any, and (f) indication of the association of the individual with park history.

While the national park idea dates back only seventy-five years, to the historic camp-fire of the Washburn-Langford Expedition on September 19, 1870, at the junction of the Firehole and Gibbon Rivers in the western part of the present Yellowstone National Park, the history of some of the other parks and monuments goes back much further, that of Death Valley National Monument and Yosemite National Park a full century or more, and that of St. Augustine, Cabrillo and Coronado National Monuments to the early days of the sixteenth century.

For some years after the creation of Yellowstone National Park in 1872, the reservation was in charge of superintendents appointed by the Secretary of the Interior, but in 1886 a troop of cavalry was detailed by the Secretary of War, upon request of the Secretary of the Interior, with the duty of patrolling the park, and in 1890 similar details of troops were made to Yosemite, Sequoia and General Grant National Parks. The military administration of the parks continued until 1914.

During the fourteen years that Mr. Mather was in charge, the various scattered areas were welded into a well-organized bureau, in accordance with the provisions of the National Park Service Act of 1916, park standards were adopted, the system of park concessions was reorganized, troops were removed and protection provided by park rangers appointed from civil life. The Tiogo Road across the Sierra was opened to travel without payment of tolls, funds were raised to preserve certain big tree groves in Sequoia National Park, then in danger of destruction by lumbering interests, a ranger service hall was established in Yosemite National Park and a system of park to park highways was provided to facilitate travel by persons who wished to visit several of the parks without the inconvenience of making long detours. New and attractive publications were issued and lectures by park naturalists were arranged to assist visitors in appreciating the full meaning of the various points of interest. Much progress was also made in the elimination of private holdings in several of the parks. These are only a few of the things that Mr. Mather accomplished and by which he will be remembered in the future.

The "Mather Collection" is only one of several methods of visualizing and summarizing the great work of conservation, interpretation and education which the National Park Service is carrying on under benefit of all of the people.

T. S. PALMER

WASHINGTON, D. C.

THE HOUSSAY JOURNAL FUND

A FEW months ago you were kind enough to insert in *SCIENCE* a brief notice of the attempt of our committee to collect a fund of money for subscriptions to all important American journals in the broad field of physiology and biology for Professor B. A. Houssay, of Buenos Aires. In addition to the note in *SCIENCE*, all members of the Federation of American Societies for Experimental Biology were circularized. The response to this plea has been generous, for 247 donations were received and a fund of \$1,543.28 was secured. This has enabled the committee to send Professor Houssay five years' subscriptions to the eighteen journals he desired, namely:

Archives of Biochemistry
American Journal of Medical Sciences
American Journal of Physiology
Anatomical Record
Annals of Internal Medicine
Archives of Internal Medicine
Biological Abstracts

Endocrinology and Journal of Clinical Endocrinology
The Journal of Clinical Investigation
Journal of Laboratory and Clinical Medicine
Journal of Neurophysiology
Journal of Nutrition
Journal of Biological Chemistry
Journal of Pharmacology and Experimental Therapeutics
Nutrition Reviews
Physiological Reviews
SCIENCE

and a sum of \$625.90 for subscriptions to foreign periodicals.

The committee takes this means of thanking the various donors for their generous participation in helping our Argentinian colleagues at a time when they were in sore need.

Committee on Houssay Journal Fund,

HERBERT M. EVANS, *Chairman*

WALTER B. CANNON

JOHN F. FULTON

CARL J. WIGGERS

SCIENTIFIC BOOKS

STATISTICAL ANALYSIS

Statistical Analysis in Biology. By K. MATHER, with a foreword by R. A. FISHER. Nine diagrams. New York: Interscience Publishers, Inc. 1943. \$4.50.

THE statistical method is an important tool in a wide range of substantive fields. While the basic procedures are not limited in their applications, the beginner prefers to learn them from a text in which they are applied directly to his own subject-matter. The present volume is by a geneticist for geneticists and other experimental biologists. It begins with the relation between sample and population. Books which open with the problem of statistical inference usually emphasize the contributions of R. A. Fisher and his associates. Mather's book falls in this group. Following a discussion of probability, significance and the basic random sampling distributions, he continues with tests of significance, the analysis of variance, experimental design, relations of two variables, the analysis of frequency data, and concludes with estimation and information.

Some biologists are disturbed by the omission of statistical derivations and mathematical proof, as in Fisher's "Statistical Methods for Research Workers." They will have little complaint with the present book on this score. Mather's approach is primarily algebraic with some elementary calculus. He shows how most of the standard equations can be derived, although those for the basic distributions, including the tests of significance, are stated without proof. In

developing the analysis of variance, randomized blocks and similar topics, he inverts the usual order. Starting with combinations and permutations, individual degrees of freedom and their variances are isolated, even though they may be added later to obtain mean squares with several degrees of freedom. In the reviewer's experience many biologists are content with learning how to use statistical equations and enough of their logic to avoid misapplying them. Mather's approach tends to bury the essential simplicity and logic of some of the techniques under the derivation of equations.

Most of the examples in the present volume are new in text-books. Perhaps two thirds of them are genetic, with most of the remainder physiological or agricultural. Some of his topics occur rarely in text-books, such as the calculation of polynomial coefficients, the discriminant function, the method of maximum likelihood, the limitations of inefficient statistics and an extended discussion of the partitioning of χ^2 .

On the debit side, Mather uses N instead of n for degrees of freedom, which is the reverse of the convention to which many of us have become adjusted. Symbolism is not consistent through the book, although this is as difficult to attain as complete freedom from errors. Many calculations are carried to several more decimal places than have any meaning. Some statements are open to question, such as the one that "neither χ^2 nor the normal deviate should ever be used when any class frequency has an expectation of 5 or less." Cochran has shown that, in

many applications, smaller expected frequencies still lead to satisfactory tests of significance. Data on the growth of maize are fitted with a polynomial curve, although the equation has no obvious biological interpretation. Because they are easy to handle statistically, polynomial curves are often computed without regard to their physical meaning. Their chief descriptive value is in indicating how many fitted constants may be needed in a rational equation which would define the relation. These limitations have been overlooked. A number of misprints have crept into the text, sometimes into equations. Presumably, they will be corrected when the book is reprinted. It is to be hoped that later editions will include some of the important topics which have been omitted, such as the analysis of experiments with missing values, transformations to stabilize the variance, the χ^2 test for homogeneity of the variance, tests for normality and a more critical discussion of the errors which are pertinent for the different comparisons of a complex experiment.

C. I. BLISS

NEW HAVEN, CONN.

ANTIBIOTIC AGENTS

Penicillin and Other Antibiotic Agents. By WALLACE E. HERRELL. 348 pp. Philadelphia and London: W. B. Saunders Company. 1945. Price \$5.00.

DURING the last five years, penicillin has attracted world-wide attention. Ever since its discovery and description by Sir Alexander Fleming, of St. Mary's Hospital in London, in the year 1929, there has been some interest in this substance, but it required the stimulus of a war to develop it to its present stage. The demonstration by Florey and his group of associates that penicillin could be produced in a form that was non-toxic to man, and that it could be used effectively in the treatment of staphylococcal infections, stimulated others to study this substance further. The development of penicillin from a laboratory curiosity to the present stage of mass production has been one of the great scientific achievements of our time. It is one of the outstanding examples of collaborative efforts on the part of governmental agencies, private industry and university and hospital personnel and laboratories. The results speak for themselves. There is no drug that can do as much for so many different infections and cause no harm to the patient. When one considers this agent is effective against the two most prevalent genitoinfectious diseases, as well as many disorders caused by gram positive microorganisms, it is possible to classify penicillin as a truly remarkable drug.

In this monograph, Dr. Herrell has summed up his own experience with penicillin and reviewed the pub-

lished work of many others. There are a number of excellent illustrations and charts, and the material is well organized and presented in a manner that is pleasing to the reader. Dr. Herrell's wide experience in this field has made him peculiarly fitted to present the subject in a thoroughgoing manner.

All physicians will want to read this monograph, which gives the results of the treatment of many diseases. A wealth of material is now accumulating in such clinical disorders as war wounds, syphilis and bacterial endocarditis, and while the early published results are most impressive, it is not possible at this time to assess the final results in such diseases as bacterial endocarditis and syphilis.

This monograph, then, can not be recommended too highly. The printing is of high quality, the bibliography is comprehensive and the index is good. Finally, the content makes excellent and satisfying reading.

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WEEDS

Weeds of Lawn and Garden. By JOHN M. FOGG, JR. 215 pages. Philadelphia: University of Pennsylvania Press, 1945. \$2.50.

THERE must be thousands of victory gardeners who are taking the hard way to get acquainted with weeds. If any of them desire to know the names, the habits or the origin of our common weeds, they will find a convenient means in Dr. Fogg's book. Here they will find notes on 242 different kinds of them and for almost every kind a non-technical description and an excellent half-page illustration. Often the young stages of the weed are shown, which is an especially valuable feature for the gardener. An introductory chapter discusses why some plants are weeds, how they are so widely and quickly dispersed over the country and how they may be exterminated.

H. A. GLEASON

NEW YORK BOTANICAL GARDEN

BOOKS RECEIVED

- HOLMBOE, JÖRGEN, GEORGE E. FORSYTHE and WILLIAM GUSTIN. *Dynamic Meteorology.* Illustrated. Pp. xvi+378. John Wiley & Sons, Inc. \$4.50. 1945.
- HOLMES, HARRY N. *Qualitative Analysis; A Brief Outline.* Ninth edition, revised. Pp. vii+52. The Macmillan Company. \$1.10. 1945.
- LOWRY, H. H., Editor, for the National Research Council. *Chemistry of Coal Utilization.* Illustrated. Vol. I, pp. 920. Vol. II, pp. 921-1868. John Wiley & Sons, Inc. Two volumes, \$20.00. 1945.
- STRECKER, EDWARD A., and KENNETH E. APPEL. *Psychiatry in Modern Warfare.* Pp. viii+88. The Macmillan Company. \$1.50. 1945.
- THOMPSON, PAUL V., Editor. *University of Colorado Studies; Series D, Physical and Biological Sciences.* Pp. 55-293. The University, \$1.00.